Synthesis and Configurational Stability of (*S*)- and (*R*)-Deuteriothalidomides

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3′-Deuteriothalidomide was synthesized and found to be configurationally five times more stable than thalidomide toward racemization at physiological pH.

Key words thalidomide; isostere; deuterium

A fundamental question is whether thalidomide (**1**) is stereospecifically teratogenic. The revival of **1** in the clinical field in the 21st century has re-activated an investigation of the molecular mechanism of the teratogenicity of **1**.[1—8] Thalidomide (**1**) was popular especially for pregnant women as an effective antiemetic for morning sickness in the 1950—60s. The teratogenic side effects, leading to birth defects such as limb reduction, produced one of the most notorious medical disasters of modern medical history, and **1** was consequently withdrawn from the market in 1962. Thalidomide (**1**) possesses an asymmetric center in the glutarimide ring. Since **1** was marketed as a racemate, it was conceivable that sedative effects of **1** might be associated with one enantiomer and the unexpected teratogenic side effects might be ascribed to the other enantiomer. Twenty years after the thalidomide disaster, only (*S*)-thalidomide (**1**) was proved to be teratogenic.[9] It was then concluded that the disaster could have been avoided if only the (*R*)-isomer of **1** had been marketed. However, it is presently unclear whether any of the actions of racemic **1** can be separated out using a pure enantiomer. According to the reports, considerable chiral inversion took place after incubation of enantiomerically pure **1**. The strongly acidic hydrogen atom at the asymmetric center of **1** rapidly epimerizes under physiological conditions, rendering bioassay of the enantiomers difficult.[10—12] Therefore, elucidation of the difference of biological activities between thalidomide enantiomers previously reported is said to be difficult. Therefore, the development of non-racemizable, chiral analogues of thalidomide has attracted much attention.[13—16] 3′-Fluorothalidimide (**2**) was developed for this purpose several years ago by a member of our group and by others.[17,18] Although the fluorine atom at the asymmetric center of **2** effectively obstructs racemization, the fluorine atom also introduces substantial electronic alterations into the molecule due to its highest electron negativity of 4.0, alterations that could be expected to significantly modulate biological activity.[19] Incidentally, deuterated drugs have been noted as a new strategy for drug discovery.[20—25] They have basically the same properties as hydrogenated compounds, but are resistant to metabolism due to the higher stability of the carbon–deuterium (C–D) bond than that of the carbon–hydrogen (C–H) bond. As a result of our research designed to produce an analogue of thalidomide that is resistant to racemization and is also a close structural mimic,[26—29] we describe herein the design and synthesis of 3′-deuteriothalidomide (**3**), the most closed isostere of thalidomide (Fig. 1). Deuterium at the 3′-position of **3** is expected to effectively block the racemization of thalidomide due to its isotopic effect without major alterations of biological activities.

(*S*)-**1**: R=H; (*R*)-**2**: R=F; (*S*)-**3**: R=D (*R*)-**1**: R=H; (*S*)-**2**: R=F; (*R*)-**3**: R=D

Fig. 1. Structures of Thalidomide (**1**) and Its Fluorinated **2** and Deuterated Isosteres **3**

Results and Discussion

Optically pure 3′-deuteriothalidomide (**3**) was synthesized in four steps from known compound **4**.[17] Namely, Boc-protected **4** was deprotonated by LiHMDS in tetrahydrofuran (THF) at −78 to −40 °C for 20 min, followed by quenching of the lithium enolate with D_2O, to obtain deuterated compound **5** with 80%. The level of deuterium of **5** was determined to be 86% by means of ^{1}H-NMR. The Boc group of **5** was then removed by trifluoroacetic acid (TFA) treatment at room temperature to furnish **6**. Next, oxidation of the 6′-position of **6** with a catalytic amount of RuO_2 in the presence of excess $NaIO_4$ in a two-phase system furnished target **3** as a racemate. Finally, the enantiomers, (*S*)- and (*R*)-**3**, were obtained by chiral HPLC separation using a DAICEL chiralpak AD, eluting with ethanol. Absolute configurations of (*S*)- and (*R*)-**3** were determined by comparison of $[\alpha]_D$ values with those of (*S*)- and (*R*)-**1** ((*S*)-**1**: $[\alpha]_D^{25}$ −64 (c=2.0, *N*,*N*-dimethylformamide (DMF)), (*R*)-**1**: $[\alpha]_D^{25}$ +64 (c=2.0, DMF),[26] (*S*)-**3**: $[\alpha]_D^{24}$ −59 (c=0.85, dimethyl sulfoxide (DMSO)), (*R*)-**3**: $[\alpha]_D^{27}$ +55 (c=0.78, DMSO)) (Chart 1).

With the target molecules in hand, we first examined the deuterium–hydrogen (D–H) exchange reaction in the stereogenic center of racemic **3** to get initial information about the stability of the C–D bond in **3**. Thus, deuterated **3** was incubated in buffer solution at 37 °C with different pH values. The level of deuterium (% D) was measured after incubation for 5 and 10 h. The deuterium *vs.* hydrogen ratio in **3** was easily seen by the use of ^{1}H-NMR. The results are shown in Table 1. The ratio of the rates of exchange of deuterium to hydrogen (H/D) increased in the order of pH values: 8.5>7.9>7.6, as well as their incubation times, *i.e.*, the D/H exchange is more rapid in basic condition (Table 1, runs 1—3). It is interesting to note that the level of H/D was 0.21 even after 10 h incubation at 7.6 pH (run 1). This result implies that the rate of racemization of **3** is slower than that of **1** because the half-life of racemization of **1** is reported to be about 10 h

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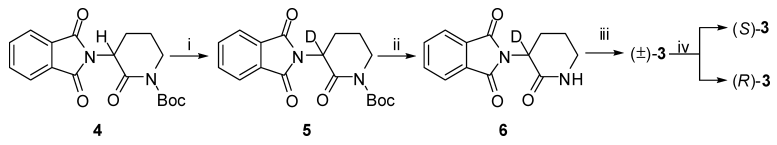

Reaction conditions: i) 1) LiHMDS (1.2 eq), THF, −78 to −40 °C, 20 min; 2) quenched by D_2O, 80%, (86% D); ii) TFA (20 eq), dichloromethane, rt, 1 h, 83%; iii) RuO_2 (0.5 eq) $NaIO_4$ (excess), water/ethyl acetate/dichloromethane, 40 °C, overnight, 95%; iv) HPLC separation by DAICEL chiralpak AD.

Chart 1

Table 1. D/H Exchange Reactions of the Racemic **3**[a)]

Run	pH	Time				
		0 h	5 h		10 h	
		% D	% D	H/D	% D	H/D
1	7.6	86	79	0.09	71	0.21
2	7.9	86	65	0.32	55	0.56
3	8.5	86	63	0.37	37	1.3

a) The levels of deuterium (% D) were measured by ^{1}H-NMR.

under neutral conditions.[11,28,29)]

These observations encouraged us to investigate the stability of optically active **1** and **3** toward racemization and hydrolysis (decay). Optically pure (*S*)-**1** and (*S*)-**3** were incubated at 37 °C and varying pH values, and monitored by HPLC. A DAICEL chiralpak AD with ethanol was used for the separation. Three-buffer systems, pH 6.18 (100 mM sodium phosphate monobasic, 100 mM sodium phosphate dibasic), pH 7.78 (40 mM Tris base, 40 mM hydrochloric acid) and pH 8.76 (40 mM Tris base, 40 mM hydrochloric acid) were employed. The results are shown in Figs. 2 and 3. In the study, (*S*)-**1** was racemized and decayed in all buffer solutions; the rates of racemization and hydrolysis were rather quick in neutral and alkaline buffer solutions, which were consistent with the earlier studies by Hashimoto as well as by us.[11,28,29)] As expected, the stability of (*S*)-**3** toward racemization is higher than that of (*S*)-**1** under all conditions. The half-life to racemization of (*S*)-**1** was estimated by a plot of the experimental data, $t_{0.5}=R/S=0.5$ while the racemization half-lives of (*S*)-**1** were found to be 31.8 h at 6.18 pH, 29.9 h at 7.78 and 3.5 h at 8.76 in buffer solution at 37 °C, those of (*S*)-**3** were 156.3 h at pH 6.18, 59.5 h at pH 7.78 and 17.9 h at pH 8.76 (Fig. 2). These results indicate that deuterated thalidomide **3** is, at least, five times as stable as thalidomide (**1**) toward racemization in buffer solutions. The effects of the pH-dependent hydrolysis of **1** and **3** are shown in Fig. 3. The hydrolysis data of **1** and **3** are essentially similar to each other in the pH 6—9 range (Fig. 3).

In summary, the first synthesis of both 3′-deuteriothalidomide (**3**) enantiomers has been accomplished from Boc-protected **4** in 4 steps including enantioseparation by chiral column chromatography.[30)] Deuterated thalidomide **3** has been found to be at least five times more stable than thalidomide (**1**) toward racemization in buffer solutions. The resistance of the enantiomers of **3** to racemization and the close structural similarity of **3** to thalidomide itself render it an excellent candidate in the search for safer thalidomide-based drugs in which the desired biological effects of **1** can be clearly separated from teratogenicity. The biological activities of **3** are now being assessed.

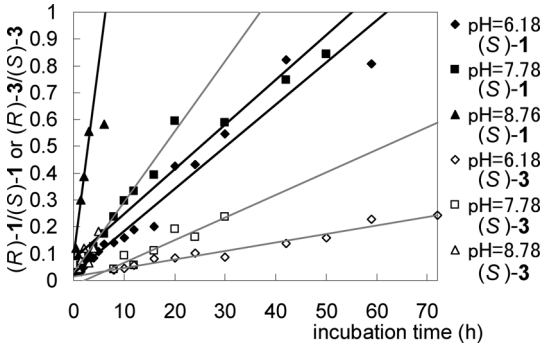

Fig. 2. Racemization Studies of (*S*)-**1** and (*S*)-**3**

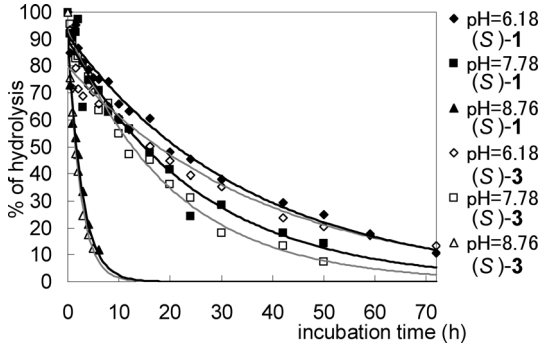

Fig. 3. Hydrolysis (Decay) Studies of (*S*)-**1** and (*S*)-**3**

Experimental

All reactions were performed in oven-dried glassware under a positive pressure of nitrogen. Solvents were transferred *via* syringe and were introduced into the reaction vessels though a rubber septum. The ^{1}H-NMR (300 MHz) spectra for solution in $CDCl_3$ were recorded on a Varian Gemini-300. Chemical shifts (δ) are expressed in ppm downfield from internal TMS (0.00 ppm). IR spectra (cm^{-1}) were recorded on a Perkin-Elmer 1600 spectrometer. EI mass spectra were taken with JEOL JMS-D300 spectrometer. (*S*)-**1** was prepared according to ref. 26.

(±)-2-(3-Deuterio-2,6-dioxo-piperidin-3-yl)-isoindol-1,3-dione (3) (3′-Deuteriothalidomide) The compound (±)-**3** was prepared from **4** according to a modified literature procedure for the preparation of 3′-fluorothalidomide (**2**).[17)] ^{1}H-NMR ($CDCl_3$) δ: 2.16 (1H, m), 2.74—2.95 (3H, m), 7.77 (2H, dd, J=3.0, 5.5 Hz), 7.90 (2H, dd, J=3.1, 5.4 Hz), 8.00 (1H, br); IR (KBr) cm^{-1}: 3193, 3097, 1776, 1698; *Anal.* Calcd for $C_{13}H_9DN_2O_4$: C, 60.23; H, 4.28; N, 10.81. Found: C, 60.00; H, 3.84; N, 10.46; HR-MS *m/z*: 259.3 (Calcd for $C_{13}H_9DN_2O_4$: 259.1).

(*S*)-2-(3-Deuterio-2,6-dioxo-piperidin-3-yl)-isoindol-1,3-dione ((*S*)-3) ((*S*)-3′-Deuteriothalidomide) $[\alpha]_D^{24}$ −59.0 (c=0.85, DMSO); HPLC (DAICEL chiralpak AD, 4.6×125 mm, MeOH=100%, flow rate 0.5 ml/min, λ=254 nm), t_R=25.34 min (major), >99% ee.

(*R*)-2-(3-Deuterio-2,6-dioxo-piperidin-3-yl)-isoindol-1,3-dione ((*R*)-3) ((*R*)-3′-Deuteriothalidomide) $[\alpha]_D^{27}$ −54.6 (c=0.79, DMSO); HPLC (DAICEL chiralpak AD, 4.6×125 mm, MeOH=100%, flow rate 0.5 ml/min, λ=254 nm), t_R=26.75 min (minor), t_R=61.67 min (major), >99% ee.

Incubation Experiments of 1 and 3 A stock solution was prepared by

dissolving (*S*)-**1** or (*S*)-**3** (20.0 mg) in dimethylsulfoxide (100 ml). The 5 ml of the stock solution was diluted with 9.5 ml of buffer (phosphate buffers for pH 6 and 7, Tris–HCl buffers for pH 8 and 9); the mixture was incubated at 37 °C. Racemization was monitored by HPLC (DAICEL chiralpak, 4.6× 250 mm, EtOH=100%, flow rate 0.5 ml/min, λ=254 nm) at regular intervals. Racemization rate was obtained to plot with incubation time as abscissa and *R*/*S* area ratio as ordinate. Hydrolysis rate was obtained to plot with incubation time as abscissa and $(R+S)$ area/(R_0+S_0) area ratio as ordinate. (R_0 and S_0 area is peak area of incubation time 0.0 h.)

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30) Although a synthesis of *N*-Boc protected racemic **3** was attempted in low yield by a different method for a different purpose,[18] neither its property nor the transformation to **3** were investigated.