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Synthesis of Racemic *cis*-5-Hydroxy-3-phthalimidoglutarimide. A Metabolite of Thalidomide Isolated from Human Plasma

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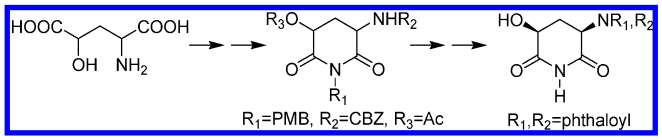

A synthesis of the glutarimide-derived metabolite of thalidomide, 5′-hydroxythalidomide (**2**), is described. The synthesis employed the lactone derivative of *N*-benzyloxycarbonyl (CBZ)-protected 4-hydroxyglutamic acid **12**, which is prepared by a de novo route from diethyl acetamidomalonate. The reaction of **12** with 4-methoxybenzylamine gave the corresponding isoglutamine, which then provided the key CBZ-protected *N*-PMB-glutarimide **14** after dehydration. Deprotection of both the CBZ and PMB groups followed by phthalimidation and deacetylation of the 3-amino-5-acetoxyglutarimide **16** afforded **2**.

In contrast to its notoriety as a potent embryotoxin, the diverse biological effects of thalidomide (**1**) have been beneficial. The compound is an approved antilepromatic and together with some of its analogues are active as immunosuppressants, immunomodulators, antiinflammatories, and anticancer agents.[1a−d] As an experimental therapeutic, thalidomide and closely related analogues have gained much attention as angiogenesis inhibitors.[1a,e,f] The antiangiogenic activity of **1** is a consequence of metabolic activation as demonstrated by in vivo studies in the rabbit and ex vivo studies with human microsomes.[2,3] At present, the identity of the active species has not been confirmed and is the objective of many synthetic and biological studies.[4−6] While the biotransformation of thalidomide to its oxygenated analogues is somewhat understood, the complete identification of the products is further complicated by its hydrolytic and configurational lability.[7] Among the suspected active components are those which result from metabolic oxygenation of the glutarimide (**2**) or phthalimide rings (**3**−**5**) and have intact imide bonds (Figure 1). The phthalimide ring-oxygenated metabolites of thalidomide (**3**−**5**) have been identified since the early investigations of its embryotoxicity and possess little or no teratogenic activity. More recently, 5′-hydroxythalidomide (**2**) was detected in plasma samples after administration of racemic **1** to healthy male volunteers and in incubates of **1** with human liver homogenate.[6a,b,c] The metabolic formation of **2** can result in the diastereoisomeric *cis* and *trans* forms which possess epimerization properties similar to those of the parent molecule.[6c] We have explored several syntheses of **2** with the goals of obtaining sufficient quantities for biological evaluation and establishing general stereoselective routes to glutarimide-derived analogues of **1**.[5] Our success with the use of 4-methoxybenzylamine as an "ammonia equivalent" in glutarimide formation in the synthesis of the active phthalimidine analogue EM-12 (**6**) has prompted our present report on the synthesis of **2** with full experimental details.[8] The synthesis described herein parallels the first synthesis of 5′-hydroxythalidomide as reported by Eger in which **2** was of interest as a tumor necrosis factor (TNF-α) inhibitor.[4] The Eger route employed glutarimide formation from *N*-benzyloxycarbonyl (CBZ)-protected 4-hydroxyisoglutamine **13** followed by CBZ deprotection and *N*-phthaloylation as the key steps. Isoglutamine **13** was prepared by amination of the CBZ-protected lactonic acid form of the rare racemic 4-hydroxyglutamic acid **9** (Scheme 1).[9, 10]

The synthesis commences with the base-mediated alkylation of commercially available diethyl acetami-

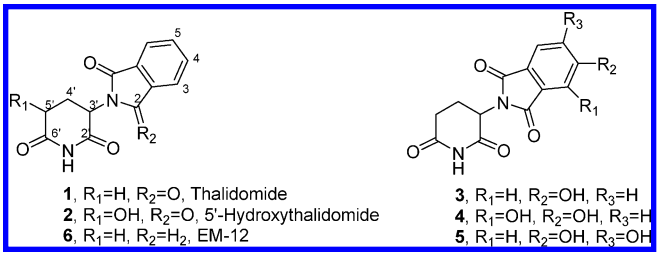

FIGURE 1. Thalidomide and analogues with varying degrees of oxygenation.

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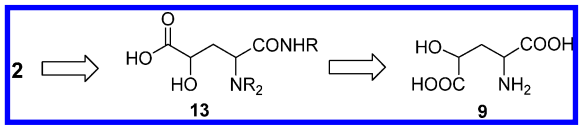
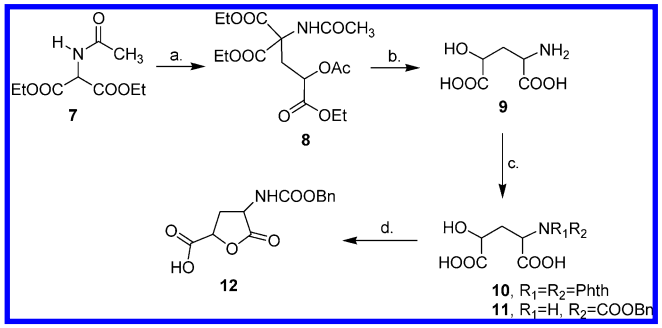
SCHEME 1. Retrosynthesis of 5′-Hydroxythalidomide 2



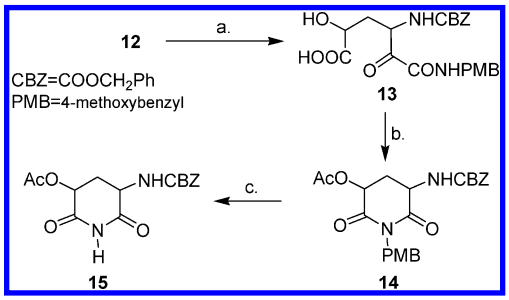
SCHEME 2. Preparation of the *N*-CBZ-lactonic Acid 12[a]



[a] Reagents and conditions: (a) NaH/THF/rt then EtOOCCH(OAc)CH_2Cl/70 °C/2 h, 88%; (b) 4 N HCl/reflux/4 h, 60%; (c) BnOCOCl/$NaHCO_3$/H_2O/20 °C/24 h, 94%; (d) 1 N HCl/100 °C, 71%.

domalonate **7**, an operation in which a successful outcome depended critically on the choice of nonprotic reaction conditions rather than the usual alcohol/alkoxide protocol (Scheme 2).[11] Following formation of the diester anion with sodium hydride in tetrahydrofuran, treatment with ethyl 2-acetoxy-3-chloropropionate gave the crystalline amidic acetoxy triester **8** in 88% yield as the exclusive product.[9a,10] Exhaustive hydrolysis of the amidic triester **8** with 4 N refluxing HCl for 16 h afforded γ-hydroxyglutamic acid **9** as a mixture of diastereoisomers. Interestingly, direct phthalimidation of the hydroxyamino acid **9** using several reagent systems based on either phthaloyl dichloride or phthalic anhydride under several sets of conditions yielded no 2-*N*-phthalolyl-4-hydroxyglutamic acid **10**, the so-called "hydroxy PG acid."[12] Treatment of **9** with benzyl chloroformate (1.5−2 equiv) in the presence of aqueous sodium bicarbonate provided the *N*-CBZ-protected hydroxyglutamic acid **11** in 94% yield as a very hygroscopic amorphous solid. Glutarimide formation by amidation−dehydration first required the preparation of

SCHEME 3. Amination of the Lactonic Acid 12 and Glutarimide Formation[a]



[a] Reagents and conditions: (a) 4-$MeOC_6H_4CH_2NH_2$/pyr/70 °C/16 h, 85%; (b) Ac_2O/$(F_3CCO)_2O$/pyr/70 °C/16 h, 68%; (c) CAN/CH_3CN/H_2O/25 °C/3 h, 68%.

the requisite *N*-CBZ-protected lactonic acid **12**. On exposure to air, **11** becomes a sticky intractable gum which is not convenient to handle or store. Converting **11** to its corresponding lactonic acid **12** leads to a crystalline solid and furthermore activates the α-carboxy group toward regiospecific isoglutamine formation when treated with amines prior to glutarimide closure. Therefore, treatment of the *N*-CBZ-protected 4-hydroxyglutamic acid **11** with boiling 1 N aqueous hydrochloric acid yielded several crops of the lactonic acid **12** in 71% combined yield. In contrast to the established use of the 4-methoxybenzyl (PMB) group as a protecting group for amides,[13a] it has not been used as a "protecting group" for imides per se other than for elimination or replacement of the acidic imide proton.[13b] Amidation of the lactonic acid **12** with 4-methoxybenzylamine in the presence of excess anhydrous pyridine furnished the *N*-CBZ-protected 4-hydroxyisoglutamine **13** in 85% yield as a white solid (Scheme 3). Treatment of isoglutamine **13** with acetic anhydride, pyridine, and a catalytic amount of trifluoroacetic acid resulted in glutarimide formation and thus provided the 5-acetoxy-(1-*N*-PMB)-2-*N*-CBZ-glutarimide **14** in 68% yield as an inseparable diastereoisomeric mixture (65:35 *cis/trans*). Interestingly, removal of the CBZ group of **14** by catalytic hydrogenolysis could not be effected to furnish the *N*-PMB-5-acetoxyaminoglutarimide **18**. Presumably, the presence of the PMB group interfered with the CBZ cleavage of **14**. The *N*-4-methoxybenzyl group was removed from **14** using ceric ammonium nitrate (CAN) in aqueous acetonitrile and provided the *N*-CBZ-5-acetoxyglutarimide **15** in 68% yield as a mixture of diastereoisomers (70:30 *cis/trans*). Deprotection−phthaloylation of the α-nitrogen of *N*-CBZ-glutarimide **15** could be accomplished in a combined two-step sequence (Scheme 4). Hydrogenation of **15** with 10% palladium on activated carbon in THF removed the CBZ group and provided the crude 3-amino-4-acetoxyglutarimide **16**. Immediate admixture of **16** with phthalic anhydride and triethylamine followed by heating resulted in *N*-phthaloylation and gave 5′-acetoxythalidomide **17** in 46% yield (from **15**) as a chromatographically inseparable mixture of diastereoisomers (65:35 *cis/trans*). While *N*-phthaloylations of aminoglutarimides such as **16** have been accomplished by heating

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(10) The preparation of **8** en route to **9** has been reported. However, important analytical details, e.g., mp and ^1H and ^{13}C NMR, were not included (ref 9b).

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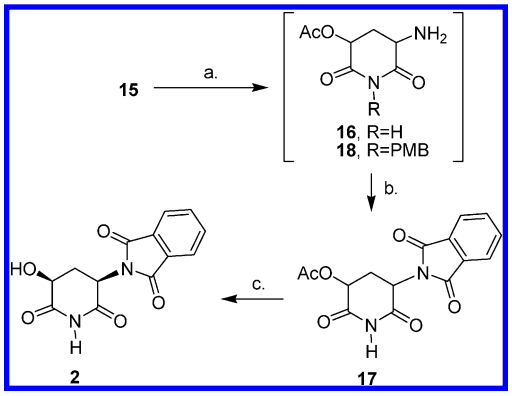
SCHEME 4. Conversion of 5-Acetoxy-2-*N*-CBZ-glutarimide 15 to 5′-OH-thalidomide 2[a]



[a] Reagents and conditions: (a) H_2/Pd/THF/20 °C/3 h, then (b) phthalic anhydride/TEA/reflux/4 h, 46% (from 15); (c) Dowex-H^+/MeOH/reflux/2 h, quant.

the substrates with phthalic anhydride in acetic acid, the same reactions in our hands resulted in both decomposition and mixtures of both 5′-acetoxythalidomide **17** and **2** (19%). The appearance of **2** is presumed to be through a hydrolytic side reaction during the phthaloylation in acid and does not proceed to completion. The deacylation of the 5′-oxygen in either the glutarimide series (such as **17**) or the analogous 4-oxygen in the acyclic glutamate series has been consistently problematic due to the sensitivity of the *N*-phthaloyl group to weakly basic conditions as well as the ease of the system to racemize.[5] On a considerably smaller scale than previously reported,[4] the methanolic *p*-toluenesulfonic acid-mediated hydrolysis of **17** provided **2** in 25% isolated yield along with decomposition products. Similarly, treatment of **17** with potassium carbonate in methanol provided **2** in 31% yield along with hydrolysis products. The best results were obtained by exposing **17** to acidic Dowex resin while heating in methanol thereby affording quantitative conversion to **2** in a *cis*/*trans* ratio of 28:1 (vide infra).

The propensity of thalidomide to racemize in biological fluids has been established, and this process, prior to metabolism, results in a diastereoisomeric mixture of *cis*- and *trans*-5′-hydroxythalidomides. High-performance liquid chromatography has proven effective in earlier investigations where the glutarimide-derived metabolites were detected, and more recently, the HPLC separation and confirmation of the individual diastereoisomers generated in vitro has been reported.[6c] The preparation of **17** consistently provides *cis*/*trans* diastereoisomeric mixtures (2:1) as determined by ^{1}H NMR. However, when the acid-mediated hydrolysis step in the conversion of **17** to **2** is undertaken under several sets of conditions, extensive epimerization takes place and the *cis*/*trans* ratio is increased to 28:1 as detected by the HPLC method that we developed for thalidomide derivatives (see the Supporting Information).

In conclusion, we have outlined an improved synthesis of the rare thalidomide metabolite 5′-hydroxythalidomide with full experimental and analytical details. The synthesis utilized a reliable and scalable multigram preparation of the intermediate *N*-CBZ-hydroxyglutamic acid and employed 4-methoxybenzylamine to introduce the glutarimide nitrogen, thereby avoiding the use of methanolic ammonia. Of particular note are the mild conditions utilized for the removal of protecting groups as well as their stability. The PMB group, while being stable to hydrogenolysis conditions, was otherwise cleaved using ceric ammonium nitrate without affecting the glutarimide ring. The removal of the 5′acetoxy group was facilitated with Dowex acid resin without cleavage of the *N*-phthaloyl group, glutarimide ring, or decomposition of the sensitive substrate.

Experimental Section

α-(4-Methoxybenzylamido)-*N*-benzyloxycarbonyl-γ-hydroxyisoglutamine (13). A mixture of *N*-benzyloxycarbonyl-γ-hydroxyglutamic acid lactone **12** (141.4 mg, 0.51 mmol) and 4-methoxybenzylamine (125 mg, 0.99 mmol) was stirred in dry pyridine (2 mL) at 70 °C for 16 h. The reaction mixture was cooled to rt followed by evaporation of the solvent. The residue was dissolved in EtOAc (10 mL), and the resulting organic phase was washed with 5% aqueous HC1 (3 × 10 mL), deionized water, (10 mL), andbrine (10 mL), dried over sodium sulfate, and concentrated. Purification of the crude residue by flash column chromatography (hexane/EtOAc, 4:1) yielded **13** (180 mg, 85%) as a white hygroscopic solid: R_f 0.67 (*n*-butanol/methanol/benzene/water, 2/4/2/2); mp 143−144 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.84 (m,1H), 1.96 (m, 1H), 3.73 (s, 3H), 3.98 (m, 1H), 4.22 (m, 3H), 5.05 (s, 2H), 6.86 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 8.3 Hz, 2H), 7.37 (m, 5H), 7.48 (d, J = 8.3 Hz, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.8, 36.6, 42.2, 52.5, 55.7, 66.2, 67.2, 79.9, 114.3, 128.4, 129.0, 132.1, 137.6, 156.7, 158.8; IR (KBr, cm^{-1}) 3317, 1697, 1651, 1535, 1253, 1100; HMRS (FAB + Na, 70 eV) calculated for $C_{21}H_{24}N_2O_7$ ([M + Na]$^+$) 439.1481, found 439.1482.

1-(4-Methoxybenzyl-3-*N*-benzyloxycarbonyl-5-acetoxypiperidine-1,6-dione (14). α-(4-Methoxybenzylamido)-*N*-benzyloxycarbonyl-γ-hydroxyisoglutamine **13** (244 mg, 0.59 mmol) was stirred in pyridine (1.2 mL) and acetic anhydride (1.2 mL) in the presence of trifluoroacetic anhydride (50 μL) at 70 °C for 16 h. The reaction mixture was cooled to room temperature followed by removal of the solvent. The residue was dissolved in EtOAc (10 mL), and the resulting solution was washed with aqueous HC1 (3 × 10 mL), deionized water (10 mL), and brine (10 mL), dried over sodium sulfate, and concentrated. Purification of the resultant crude residue by flash column chromatography (hexane/EtOAc, 4:1) yielded **14** (175 mg, 68%; 65:35, *cis*/*trans*) as a hygroscopic white solid: R_f 0.53 (hexane/ethyl acetate, 1:1); mp 46−50 °C; ^{1}H NMR (DMSO)-d_6, 500 MHz) δ 2.10 (s, 3H), 2.28 (m, 2H, 4-H), 3.71 (s, 3H), 4.53 (dd, J = 7.6 Hz, 14.4 Hz, 1H, 3-H *trans*), 4.75 (m, 3H, CH_2 + 3-H *cis*), 5.07 (s, 2H), 5.70 (dd, J = 5.5 Hz, 7.0 Hz, 1H, 5-H *trans*), 582 (dd, J = 6.8 Hz, 11.7 Hz, 1H, 5-U *cis*),6.85 (d, J = 8.3 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.37 (m, 5H), 7.82 9 (d, J = 8.7 Hz, 1H, NH *cis*), 8.10 (d, J = 8.9 Hz, 1H, NH *trans*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.1, 29.9, 43.5, 43.5, 49.2, 50.5, 55.71, 55.77, 57.8, 66.3, 67.6, 68.4, 111.0, 128.49, 128.59, 129.09, 129.48, 129.86, 137.5, 156.7, 159.09, 159.11, 169.86, 169.93, 170.0, 171.9; HMRS (FAB + Na, 70 eV) calculated for $C_{23}H_{24}N_2O_7$ ([M + Na]$^+$) 463.1481, found 463.1494.

3-*N*-Benzyloxycarbonyl-5-acetoxypiperidine-1,6-dione (15). 1-(4-Methoxybenzyl)-3-*N*-benzyloxycarbonyl-5-acetoxypiperidine-1,6-dione **14** (175.8 mg, 0.4 mmol) was stirred in a mixture of acetonitrile/water (3:1) and ammonium cerium(IV) nitrate (859 mg, 1.6 mmol) at rt for 3 h. The reaction mixture was quenched by addition of saturated aqueous sodium thiosulfate (2 mL) and saturated aqueous sodium carbonate (2 mL), which led to the formation of a thick, white precipitate. The reaction mixture was then filtered through a short Celite pad and the filtrate was concentrated to a white/yellow solid residue. The residue was submitted to flash column chromatography (hexane/EtOAc, 2:1) and gave **15** as a white solid (87 mg, 68%; 70:30 mixture of *cis* and *trans*): R_f 0.23 (hexane/ethyl acetate,

1:1); mp 176−178 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.10 (s, 3H), 2.22 (m, 2H, 4-H), 4.38 (dd, J = 7.7 Hz, 14.2 Hz, 1H, 3-H *trans*), 4.62 (m, 1H, 3-H *cis*), 5.06 (s, 2H), 5.55 (dd, J = 5.1 Hz, 7.5 Hz, 1H, 5-H *trans*), 5.69 (dd, J = 6.0 Hz, 12.6 Hz, 1H, 5-H *cis*), 7.36 (m, 5H), 7.69 (d, J = 8.7 Hz, 1H, CONH *cis*), 7.98 (d, J = 8.8 Hz, 1H, CONH *trans*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 20.5, 29.9, 48.2, 49.7, 65.63, 65.83, 66.7, 67.5, 127.79, 127.88, 128.3, 136.73, 136.84, 155.9, 156.0, 169.19, 169.27, 169.83, 170.8, 171.7; IR (KBr, cm^{-1}) 3300, 1740, 1693, 1531, 1373, 1219, 1037; HMRS (FAB + Na, 70 eV) calcd for $C_{15}H_{16}N_2O_6$ ([M + Na]$^+$) 343.0906, found 343.0910.

3-*N*-Phthaloyl-5-acetoxypiperidine-1,6-dione (5′-Acetoxythalidomide) (17). 3-*N*-Benzyloxycarbonyl-5-acetoxypiperidine-1,6-dione **15** (101.3 mg, 0.32 mmol) was stirred in dry THF (3.3 mL) under hydrogen gas (1 atm) in the presence of palladium over activated carbon (10.1 mg, 10 wt % of substrate) for 3 h. The reaction mixture was then filtered through a short Celite pad followed by concentration of the filtrate to about 3.5 mL. The resultant unprotected aminoglutarimide was then directly mixed with phthalic anhydride (56.2 mg, 0.38 mmol) and triethylamine (140 μL, 1.00 mmol) and refluxed (4 h). TLC (*n*-butanol/methanol/water/benzene, 2:4:2:2) of the reaction mixture showed full conversion of the unprotected amine to the chromatographically more mobile product. The reaction mixture was cooled to room temperature and the solvent was removed. The crude residue was purified by flash column chromatography (hexane/EtOAc, 1:1) to yield **17** as a 65:35 mixture of *cis* and *trans* diastereoisomers (white solid) (45.6 mg, 46%): R_f 0.32 (hexane/EtOAc, 1:1); mp 251−253 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.12 (s, 3H, AcO *cis*), 2.13 (s, 3H, AcO *trans*), 2.41 (m, 1H, 4-H), 2.43 (m, 1H, 4-H), 2.67 (m, 1H,- 4-H), 2.70 (m, 1H,4-H), 5.10 (t, J = 7.0 Hz, 1H, 5-H *trans*), 5.48 (dd, J = 5.4 Hz, 13.2 Hz, 1H, 5-H *cis*), 5.63 (dd, J = 5.1 Hz, 7.9 Hz, 1H, 3-H *trans*), 5.86 (dd, J = 5.6 Hz, 13.1 Hz, 1H, 3-H *cis*), 7.9 (m, 4H), 11.55 (s, 1H, NH *cis*), 11.65 (s, 1H, NH *trans*); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.1, 28.28, 28.55, 46.5, 48.4, 67.5, 68.1, 124.11, 124.23, 124.40, 131.88, 131.92, 135.7, 167.9, 169.88, 169.92, 170.3; IR (KBr,cm^{-1}) 3800, 1712, 1392, 1227, 1088; HMRS (FAB + Na, 70 eV) calcd for $C_{15}H_{12}N_2O_6$ ([M + Na]$^+$) 339.0593, found 339.0600.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of compounds **8**, **9**, **11**−**15**, and **17**, the HPLC chromatogram of compound **2,** and experimental procedures for compounds **8**, **9**, **11**, **12**, and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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