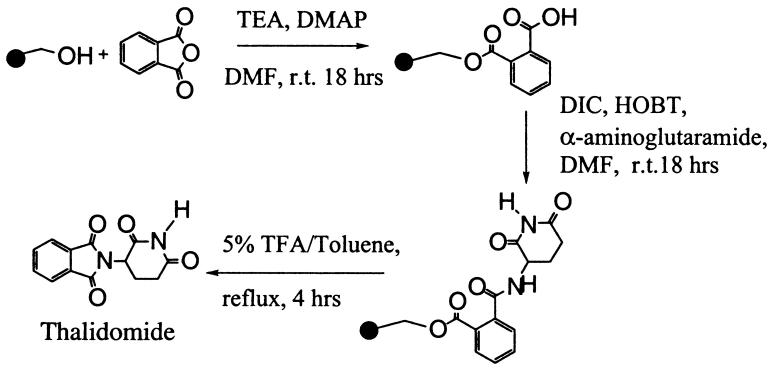
JOURNAL OF combinatorial CHEMISTRY

Article

Solid-Phase Synthesis of Thalidomide and Its Analogues

Zili Xiao, Kevin Schaefer, Steven Firestine, and Pui-Kai Li

J. Comb. Chem., **2002**, 4 (2), 149-153• DOI: 10.1021/cc010038n • Publication Date (Web): 05 February 2002



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

Solid-Phase Synthesis of Thalidomide and Its Analogues

Zili Xiao,† Kevin Schaefer,† Steven Firestine,‡ and Pui-Kai Li*,†

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210, and Department of Medicinal Chemistry, Mylan School of Pharmacy, Duquesne University, Pittsburgh, Pennsylvania 15282

Received June 27, 2001

A novel solid-phase synthesis of thalidomide and its metabolites and analogues is described. The synthetic strategy involves the coupling of hydroxymethyl polystyrene with phthalic anhydride to form the resin-linked acid. The acid is then reacted with primary amines followed by acid or base treatment to form thalidomide and its analogues with either open or closed phthalimide rings. Most of the analogues are synthesized with high yields (40.3−98.1% in three steps) and purities (92.3−98.9%).

Introduction

It has been shown that thalidomide, a sedative and hypnotic drug best known for its severe teratogenic potential, inhibits angiogenesis.[1] Angiogenesis is involved in adult human physiological processes such as pregnancy, menstruation, and wound healing[2] and in pathological processes such as tumor growth and metastasis.[3,4] In this last respect, thalidomide has been shown to reduce tumor growth by blocking angiogenesis both in model systems and in clinical studies.[5−8] Thalidomide is a prodrug, and metabolism of thalidomide is critical to its antiangiogenic activity.[9] Because the antiangiogenic activity of thalidomide was reported recently,[1] only a limited number of thalidomide metabolites have been tested for their antiangiogenic activity. Figure 1 shows the known and hypothesized metabolites of thalidomide.[10−16] Thalidomide can be metabolized through hydrolysis at the glutarimide and/or phthalimide ring to yield metabolites M1−M3, M6−M11, and M14−M15. Compounds M4, M5, M12, and M13 can be considered as the decarboxylated analogues of M2, M3, M11, and M9, respectively. In addition to hydrolysis, hydroxylation can occur on glutarimide or phthalimide ring to give metabolites M16−M21. Interestingly, only metabolites M3 (phthaloylglutamic acid) and M16 (4-OH thalidomide) have been tested for their antiangiogenic activities.[1,17] Recently, we have synthesized and tested several metabolites of thalidomide. Several hydroxylated metabolites of thalidomide exhibited antiangiogenic activities. In addition, substituting the glutarimide ring of thalidomide with substituted phenyl groups resulted in potent antiangiogenic agents. Currently, the mechanism of antiangiogenic action and the identity of the active antiangiogenic moiety or moieties of thalidomide are still unknown. We are interested in mapping the active antiangiogenic pharmacophore of thalidomide. To generate a pharmacophore of thalidomide, an efficient high-throughput screening method for antiangiogenic agents and a combinatorial library of thalidomide analogues are needed. Here, we report an effective solid-phase synthesis of thalidomide analogues. This solid-phase synthetic procedure can be used to synthesize a combinatorial library of thalidomide analogues.

* To whom correspondence should be addressed. Phone: (614) 688-0253. Fax: (614) 688-8556. E-mail: li.27@osu.edu.

† The Ohio State University.

‡ Duquesne University.

Results and Discussion

The classical solution-phase method for the synthesis of phthalimide involves reacting an amine with a phthalic anhydride, followed by ring closure. Solvents used are pyridine, toluene/triethylamine,[18] or acetic acid at reflux temperature.[19] The ring-closure step is usually unreliable, and purification of products is required. Solid-phase synthesis allows for the synthesis of a large number of analogues of thalidomide as well as the generation of a combinatorial library. We first attempted to synthesize thalidomide using a solid-phase synthetic approach (Figure 2). The first step was involved the coupling of hydroxymethyl polystyrene **1** with phthalic anhydride **2** in the presence of triethylamine (TEA), 4-dimethylaminopyridine (DMAP), and dimethylformamide (DMF) to form the resin-linked acid **3**. The acid was then coupled to α-aminoglutarimide in the presence of diisopropylcarbodiimide (DIC) and *N*-hydroxybenzotriazole (HOBT) to yield the amide **4**. Cleavage of **4** from the resin with *5%* trifluoroacetic acid (TFA) in toluene at reflux simultaneously formed the phthalimide ring system to yield thalidomide with an overall yield of 69.7% (total of three steps a−c). We also investigated the possibility of hydrolyzing the ester resin linker without forming the phthalimide ring in **4.** Indeed, stirring **4** in 1% KOH/CH_3OH at room temperature for 2 h generated one of the thalidomide metabolites with a partially opened phthalimide ring, α-(*o*-carboxybenzamido)glutarimide (M8) (Figure 3) (total overall yield, 40.3%). Since thalidomide and α-(*o*-carboxybenzamido)glutarimide (M8) are derived from the same intermediate **4**, the difference in their overall yields is apparently due to the stability of the glutarimide under acidic vs basic conditions. It has been reported that the glutarimide ring in thalidomide is subjected to hydrolysis at alkaline pH.[10]

10.1021/cc010038n CCC: $22.00
Published on Web 02/05/2002

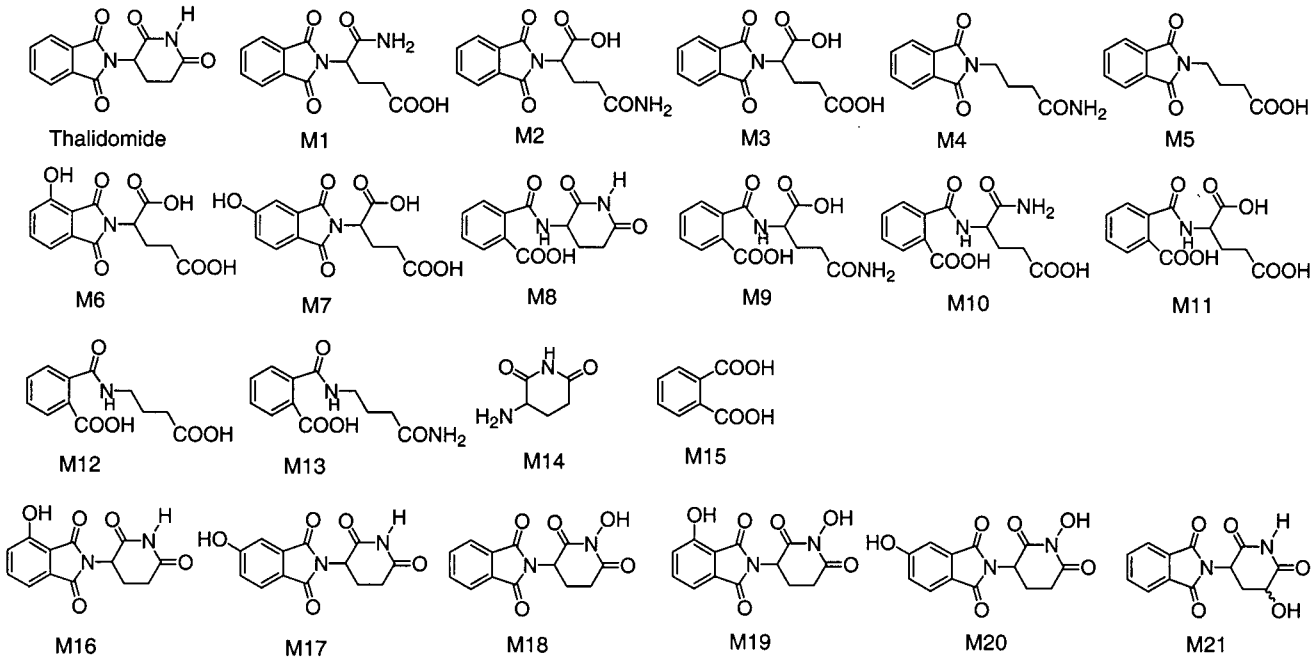


Figure 1. Structures of the metabolites and analogues of thalidomide.

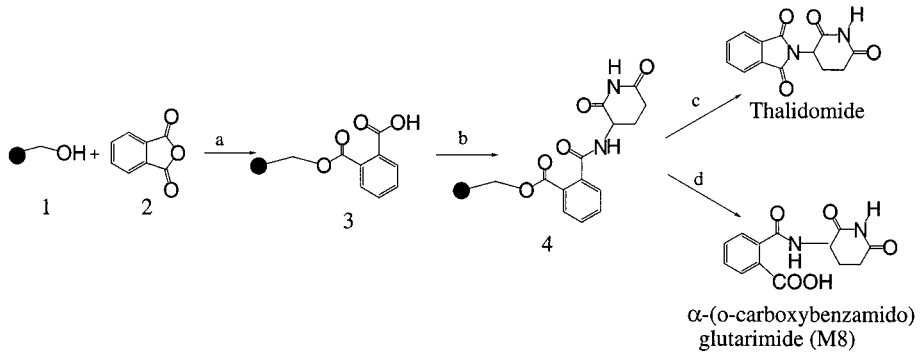


Figure 2. Solid-phase synthesis of thalidomide and α-(*o*-carboxybenzamido)glutarimide (M8). Reagents and conditions are the following: (a) TEA, DMAP, DMF, room temp, 18 h; (b) DIC, HOBT, α-aminoglutarimide, DMF, room temp, 18 h; (c) *5%* TFA/toluene, 4 h; (d) 1% KOH/CH_3OH, room temp, 2 h.

After successfully completing the synthesis of thalidomide and M8, we knew we could introduce diversity at the position occupied by the glutarimide ring. Replacing the glutarimide ring of thalidomide with phenyl and diethyl glutamate groups yielded *N*-phenylphthalimide and diethylphthaloyl glutamate, respectively (Figure 3). In M8, replacing the glutarimide ring with phenyl and diethyl glutamate groups afforded 2-[(phenylamino)carbonyl]benzoic acid and 2-(*o*-carboxybenzamido)-glutaric acid, respectively (Figure 3). The overall yields of all four compounds range from 55.1% to 98.1%. Resin-linked acid **3** is the common intermediate in the synthesis of the compounds. When **3** was coupled to aniline in the presence of DIC, HOBT in DMF yielded the amide **5**. Compound **6** was obtained in a procedure similar to the synthesis of **5** except aniline was replaced with diethyl glutamate. Acid-catalyzed cleavage from the resin with concurrent phthalimide ring formation of compounds **5** and **6** afforded *N*-phenylphthalimide and diethyl phthaloylglutamate, respectively. 2-[(Phenylamino)carbonyl]benzoic acid, an analogue of *N*-phenylphthalimide with a partially opened phthalimide ring, was obtained from **5** through basic cleavage of the resin linker at room temperature. However, the temperature of the same basic hydrolytic conditions had to be raised from room temperature to reflux in order to obtain 2-(*o*-carboxybenzamido)glutaric acid from **6** (Figure 3).

The overall yield and purity of the final compounds were determined by reversed-phase HPLC using a purified standard for comparison (Figure 4). The purity of the compounds range from 92.3% (thalidomide) to 98.9% (2-[(phenylamino)-carbonyl]benzoic acid) and are high enough for biological testing without further purification. In addition to high purity, the compounds are obtained with good yield (40.3−98.1% based on crude weight and relative to the initial loading of hydroxypolystyrene, 1.2 mmol/g) (Figure 4). The highest-yield compounds are *N*-phenylphthalimide (98.1%) and its partially opened phthalimide ring analogue 2-[(phenylamino)-carbonyl]benzoic acid (93.6%). When the yields of *N*-phenylphthalimide (98.1%) and diethyl phthaloylglutamate (55.1%) were compared, one of the possible explanations is that the increased conformational mobility of the diethyl-glutaryl group in diethyl phthaloylglutamate rendered the molecule less preorganized for cyclization. However, the low yield of α-(*o*-carboxybenzamido)glutarimide (40.3%) vs

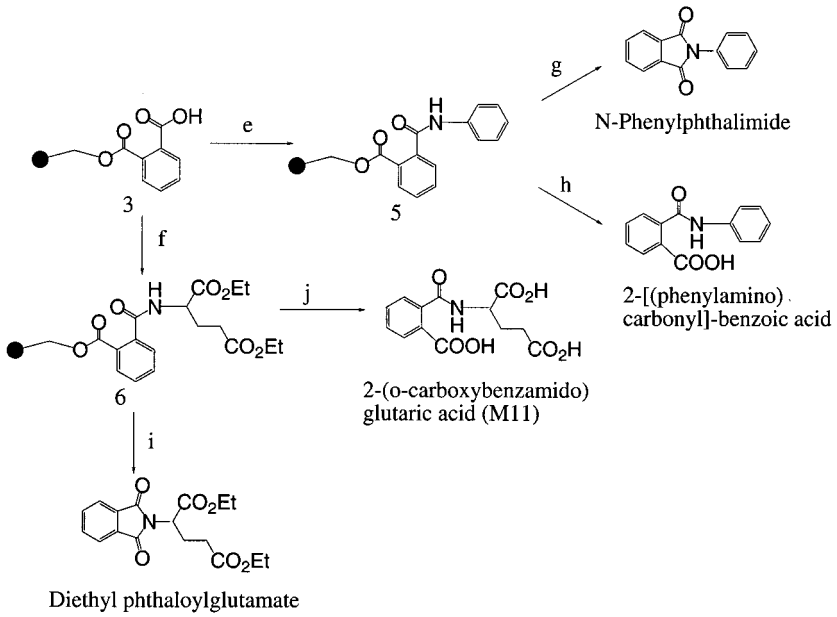


Figure 3. Solid-phase synthesis of thalidomide analogues. Reagents and conditions are the following: (e) DIC, HOBT, DMF, aniline, room temp, 18 h; (f) same as step e (replace aniline with diethyl glutamate); (g) 5% TFA/toluene, reflux 12 h; (h) 1% KOH/CH_3OH, room temp, 2 h; (i) same as step g; (j) 1% KOH/CH_3OH, reflux, 2 h.

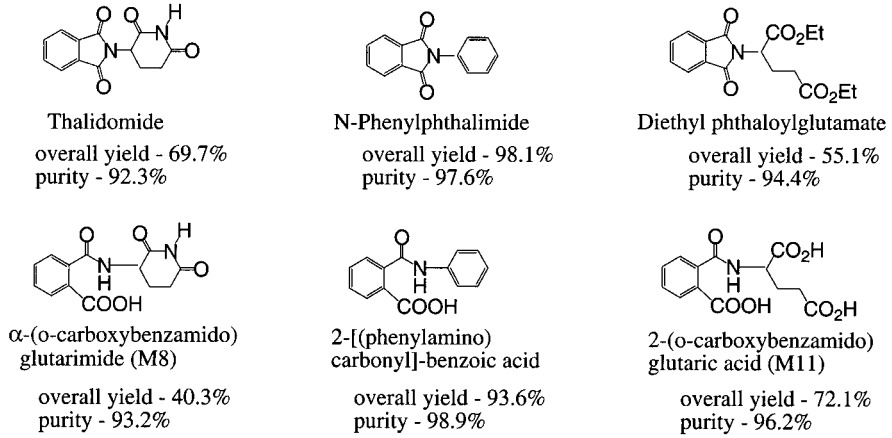


Figure 4. Percentage yield and purity of thalidomide and its analogues.

Table 1. Percentage Yield of *N*-Phenylphthalimide and Diethyl Phthaloylglutamate under Different Acid-Catalyzed Cyclization Conditions

compd	5% AcOH/toluene, room temp, 36 h (%)	5% AcOH/toluene, 60 °C, 18 h (%)	5% AcOH/DMF, 130 °C, 14 h (%)	5% TFA/toluene, reflux (%)
N-phenylphthalimide	<5	93.4	59.0	98.1

compd	5% AcOH/toluene, 90 °C, 18 h (%)	5% AcOH/toluene, reflux 18 h (%)	5% TFA/toluene, reflux, 18 h (%)
diethyl phthaloylglutamate	0	11.1	55.1

2-[(phenylamino)carbonyl]benzoic acid (93.6%) could be due to the instability of the glutaryl moiety on basic hydrolysis.

We also investigated the conditions of the acid-catalyzed cleavage of the amides **5** (step g, Figure 3) and **6** (step i, Figure 3) with simultaneous phthalimide ring-closure forming *N*-phenylphthalimide and diethyl phthaloylglutamate, respectively. Different conditions for the cleavage and cyclization were examined (Table 1). Five percent acetic acid in toluene was used initially. A very small amount of *N*-phenylphthalimide (<5%) was formed when compound **5** was stirred in 5% acetic acid in toluene at room temperature for 36 h. The yield was quantitative (93.4%) when the temperature of the reaction mixture was raised to 60 °C and stirred for 18 h. A further increase in temperature did not improve the yield. Substituting acetic acid with trifluoroacetic acid and stirring at reflux for 4 h recorded the best yield (98.1%). The same trend was also observed for the synthesis of diethyl phthaloylglutamate from amide **6** (Table 1).

Summary

In summary, the solid-phase synthesis of thalidomide and its analogues with high purity has been completed. The

success of the synthesis has now set the stage for the synthesis of a combinatorial library of thalidomide analogues, which is needed to generate an active antiangiogenic pharmacophore of thalidomide. A more detailed discussion of the antiangiogenic activity of the library will be reported in due course.

Experimental Section

General Information. All the chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA), analyzed for purity by TLC, and used as received unless otherwise indicated. Hydroxymethyl polystyrene was purchased from Calbio-Novabiochemical Corp. (La Jolla, CA). Silica gel TLC plates (60 F_{254}) were purchased from Scientific Adsorbents, Inc. (Atlanta, GA). Melting points were determined in open capillaries on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR was recorded on an Bruker dpx 250 spectrometer at 250 MHz and reported in ppm. A mass spectrum was obtained at The Ohio State University Campus Chemical Instrumentation Center on a VG 70-2505, a Nicolet FTMS-200, or a Finnigan MAT-900 mass spectrometer. The purity of the compounds was determined by HPLC (Beckman System Gold HPLC 127; Fullerton, CA) using a NovaPak C18 reversed-phase column (150 mm × 3.9 mm i.d.) (Waters Associates; Milford, MA) and an ultraviolet detector (Beckman UV detector 166) (230 nm).

Preparation of 3. To a suspension of hydroxymethyl polystyrene resin **1** (5.0 g, 6.2 mmol; Nova Biotech, 1.24 mmol/g; 100−200 mesh) in DMF (50 mL) were added triethylamine (3.13 g, 31 mmol), DMAP (0.76 g, 6.2 mmol), and phthalic anhydride **2** (4.6 g, 31 mmol). The mixture was stirred at room temperature for 18 h and filtered, and the remaining solid was washed with DMF (3 × 10 mL), DCM (3 × 10 mL), and methanol (3 × 10 mL) and then dried at 45−60 °C under reduced pressure to obtain 6.62 g of **3**.

General Procedure for the Preparation of Amides 4−6. To the mixture of **3** (1.0 mmol), amine (1.0 mmol) in DMF (5 mL), diisopropylcarbodiimide DIC (1.0 mmol), and HOBT (1.0 mmol) were added, and the mixture was stirred at room temperature for 18 h. The mixture was filtered, and the remaining resin was washed with DMF (3 × 5 mL), DCM (3 × 5 mL), and methanol (3 × 5 mL) and dried in a vacuum at 45−60 °C to generate the amides.

Preparation of α-(*o*-Carboxybenzamido)glutarimide. Compound **4** (1.25 mmol) was added to a solution of 1% KOH in methanol (10 mL). The mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, and the residue obtained was acidified with 2% HCl. The resulting mixture was extracted with ethyl acetate (3 × 30 mL), the organic layer was washed with saturated NaCl and dried (anhydrous Na_2SO_4), and the solvent was evaporated under reduced pressure to afford α-(*o*-carboxybenzamido)glutarimide: mp 132−134 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.91−2.81 (m, 4H, $-CH_2-CH_2-$), 4.68 (m, 1H, −CH−), 7.42−7.82 (m, 4H, aromatic H), 8.60 (d, J = 8.04 Hz, 1H, −N*H*CH−), 10.83 (s, 1H, −CO−N*H*−CO−), 12.95 (br s, 1H, −COOH). An analytical sample was obtained by silica gel column chromatography eluted with 5% CH_3OH in EtOAc. HPLC (CH_3OH/H_2O, 2:3): flow rate, 1 mL/min; retention time, 1.05 min; 93.2% purity. HRMS *m*/*z* (M + Na): calculated, 229.0644; found, 229.0650.

Preparation of 2-[(Phenylamino)carbonyl]benzoic Acid. The preparation of 2-[(phenylamino)carbonyl]benzoic acid is similar to the preparation of α-(*o*-carboxybenzamido)-glutarimide except compound **5** was used as the starting material: mp 167−169 °C (lit. 169 °C (dec)[20]). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 7.12−8.00 (m, 9H, aromatic H), 10.44 (s, 1H, −NH−), 13.12 (brs, 1H, −COOH). HPLC (CH_3OH/H_2O, 2:3): flow rate, 1 mL/min; retention time 1.6 min; 98.9% purity.

Preparation of 2-(*o*-Carboxybenzamido)glutaric Acid. The preparation of 2-(*o*-carboxybenzamido)glutaric acid is similar to the preparation of α-(*o*-carboxybenzamido)-glutarimide except compound **6** was used as the starting material and the mixture was refluxed for 2 h instead of being stirred at room temperature: mp 161−162 °C (lit. 168−170 °C[10]). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.71−2.52 (m, 4H, $-CH_2CH_2-$), 4.36 (m, 1H, −CH−), 7.32−7.72 (m, 4H, aromatic-H), 8.53 (d, J = 7.80 Hz, 1H, −NH−), 12.71 (brs, −COOH). HPLC (CH_3OH/H_2O, 2:3): flow rate, 1 mL/min; retention time, 1.1 min; 96.2% purity. HRMS *m*/*z* (M + Na): calculated, 318.0590; found, 318.0581.

Preparation of Thalidomide. Compound **4** (1.0 mmol) was added to a solution of 5% TFA in toluene. The mixture was refluxed for 4 h. After cooling, the mixture was filtered and the solid was washed with CH_3OH (3 × 10 mL). The filtrates were combined and evaporated to dryness under reduced pressure to obtain thalidomide: mp 273−273.5 °C (lit. 273 °C[10]). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.84−3.01 (m, 4H, $-CH_2CH_2-$), 5.14 (m, 1H, −CH−), 7.71−8.00 (m, 4H, aromatic-H), 11.12 (s, 1H, −NH−). HPLC (CH_3OH/H_2O, 3:7): flow rate, 1 mL/min; retention time, 4 min; 92.3% purity.

Preparation of *N*-Phenylphthalimide. The preparation of *N*-phenylphthalimide is similar to the preparation of thalidomide except compound **5** was used as the starting material: mp 206−207 °C (lit. 209−211 °C[21]). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 7.37−7.58 (m, 5H, aromatic-H), 7.86−8.01 (m, 4H, aromatic-H). HPLC (CH_3OH/H_2O, 1:1): flow rate, 1.25 mL/min; retention time, 5.5 min; 97.6% purity.

Preparation of Diethyl Phthaloylglutamate. The preparation of diethyl phthaloylglutamate is similar to the preparation of thalidomide except compound **6** was used as the starting material. The compound was obtained as an oil. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.06−1.38 (m, 6H, 2 × CH_3), 2.18−2.71 (m, 4H, $-CH_2CH_2-$), 3.90−4.28 (m, 4H, 2 × C*H*$_2$CH$_3$), 4.86 (m, 1H, −CH−), 7.60−7.97 (m, 4H, aromatic-H). HPLC (CH_3OH/H_2O, 1:1): flow rate, 1.2 mL/min; retention time, 8.5 min; 94.4% purity. An analytical sample was obtained by silica gel column chromatography eluted with EtOAc/CH_2Cl_2 (1:3). HRMS *m*/*z* (M + Na): calculated, 328.0797; found, 328.0803.

References and Notes

(1) D'Amato, R. J.; Loughnan, M. S.; Flynn, E.; Folkman, J. Thalidomide is an inhibitor of angiogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4082−4085.

(2) Veikkola, T.; Alitalo, K. VEGFs, receptors and angiogenesis. *Semin. Cancer Biol.* **1999**, *9*, 211−220.
(3) Folkman, J.; Ingber, D. Inhibition of angiogenesis. *Semin. Cancer Biol.* **1992**, *3*, 89−96.
(4) Gasparini, G. The rationale and future potential of angiogenesis inhibitors in neoplasia. *Drugs* **1999**, *58*, 17−38.
(5) Singhal, S.; Mehta, J.; Desikan, R.; Ayers, D.; Roberson, P.; Eddlemon, P.; Munshi, N.; Anaissie, E.; Wilson, C.; Dhodapkar, M.; Zeddis, J.; Barlogie, B. Antitumor activity of thalidomide in refractory multiple myeloma [see comments]. *N. Engl. J. Med.* **1999**, *341*, 1565−1571.
(6) Juliusson, G.; Celsing, F.; Turesson, I.; Lenhoff, S.; Adriansson, M.; Malm, C. Frequent good partial remissions from thalidomide including best response ever in patients with advanced refractory and relapsed myeloma. *Br. J. Haematol.* **2000**, *109*, 89−96.
(7) Patt, Y. Z.; Hassan, M. M.; Lozano, R. D.; Ellis, L. M.; Peterson, J. A.; Waugh, K. A. Durable clinical response of refractory hepatocellular carcinoma to orally administered thalidomide. *Am. J. Clin. Oncol.* **2000**, *23*, 319−321.
(8) Zomas, A.; Anagnostopoulos, N.; Dimopoulos, M. A. Successful treatment of multiple myeloma relapsing after high-dose therapy and autologous transplantation with thalidomide as a single agent. *Bone Marrow Transplant.* **2000**, *25*, 1319−1320.
(9) Bauer, K. S.; Dixon, S. C.; Figg, W. D. Inhibition of angiogenesis by thalidomide requires metabolic activation, which is species-dependent. *Biochem. Pharmacol.* **1998**, *55*, 1827−1834.
(10) Schumacher, H.; Smith, R. L.; Williams, R. T. The metabolism of thalidomide, the spontaneous hydrolysis of thalidomide in solution. *Br. J. Pharmacol.* **1965**, *25*, 324−337.
(11) Schumacher, H.; Smith, R. L.; Williams, R. T. The metabolism of thalidomide: the fate of thalidomide and some of its hydrolysis products in various species. *Br. J. Pharmacol.* **1965**, *25*, 338−351.
(12) Schumacher, H.; Blake, D. A.; Gillette, J. R. Disposition of thalidomide in rabbits and rats. *J. Pharmacol. Exp. Ther.* **1968**, *160*, 201−211.
(13) Eriksson, T.; Bjorkman, S.; Fyge, A.; Ekberg, H. Determination of thalidomide in plasma and blood by high-performance liquid chromatography: avoiding hydrolytic degradation. *J. Chromatogr.* **1992**, *582*, 211−216.
(14) Eriksson, T.; Bjorkman, S.; Roth, B.; Bjork, H.; Hoglund, P. Hydroxylated metabolites of thalidomide: formation in-vitro and in-vivo in man. *J. Pharm. Pharmacol.* **1998**, *50*, 1409−1416.
(15) Blaschke, G.; Hess, H. R.; Lupke, N. P. (Synthesis and teratogenic action of *n*-hydroxythalidomide). *Arzneimittelforschung* **1989**, *39*, 293−294.
(16) Meyring, M.; Chankvetadze, B.; Blaschke, G. Simultaneous separation and enantioseparation of thalidomide and its hydroxylated metabolites using high-performance liquid chromatography in common-size columns, capillary liquid chromatography and nonaqueous capillary electrochromatography. *J. Chromatogr. A* **2000**, *876*, 157−167.
(17) Kenyon, B. M.; Browne, F.; D'Amato, R. J. Effects of thalidomide and related metabolites in a mouse corneal model of neovascularization. *Exp. Eye Res.* **1997**, *64*, 971−978.
(18) Shah, J. H.; Swartz, G. M.; Papathanassiu, A. E.; Treston, A. M.; Fogler, W. E.; Madsen, J. W.; Green, J. G. Synthesis and enantiomeric separation of 2-phthalimidinoglutaric acid analogues: potent inhibitors of tumor metastasis. *J. Med. Chem.* **1999**, *42*, 3014−3017.
(19) Vamecq, J.; Bac, P.; Herrenknecht, C.; Maurois, P.; Delcourt, P.; Stables, J. P. Synthesis and anticonvulsant and neurotoxic properties of substituted *N*-Phenyl derivatives of the phthalimide pharmacophore. *J. Med. Chem.* **2000**, *43*, 1311−1319.
(20) Hawkins, M. D. Intramolecular catalysis. Part III. Hydrolysis of 3′- and 4′-substituted phthalanilic acids [*o*-(*N*-phenylcarbamoyl)benzoic acids] *J. Chem. Soc., Perkin Trans. 2* **1976**, 642−647.
(21) Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S. Phenylphthalimides with tumor necrosis factor alpha production-enhancing activity. *Chem. Pharm. Bull.* **1996**, *44*, 156−162.

CC010038N