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IMPROVEMENT OF SYNTHETIC PATHWAYS TO THALIDOMIDE ESTER DERIVATIVES

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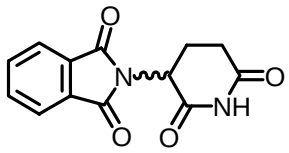
Abstract – Three synthetic approaches to *N*-substituted Thalidomide prodrugs from *(S)*-phtaloylglutamic acid, *(S)*-Boc-glutamic acid or phtaloylglutamic anhydride are described and compared. The most efficient method is a one-pot synthesis which allowed to obtain an ester derivative of Thalidomide with 67 % yield.

INTRODUCTION

Thalidomide is a drug which has been withdrawn of the market due to its teratogenic effects. More recently, it regained scientific interest because of its great therapeutic efficiency in the treatment of several diseases including leprosy,[1] rhumatoid arthritis,[2] Crohn's disease,[3] and cancer, related to pathologic angiogenesis.[4-6] For this reason, synthetic approaches to racemic Thalidomide (Figure 1) were developped. A one-pot Thalidomide synthesis was recently published using microwave irradiation.[7] A multistep synthesis was also developped using formal [3+3] cycloaddition.[8] Nevertheless, therapeutic use of Thalidomide is limited by the low solubility of this compound. To avoid this inconvenient, a series of new water-soluble *N*-substituted Thalidomide prodrugs was prepared.[9] Some of them exhibited a higher activity than Thalidomide in the inhibition of TNF-α release, and a positive corrrelation between prodrug stability and activity was found. Nevertheless, the synthetic pathways to access to this kind of products needed Thalidomide or analogs which are not commercialy available compounds. We developped a

strategy consisting in the incorporation of an amino-ester by substitution of nitrogen atom in the glutarimide ring. The purpose of this work is to present several synthetic approaches starting from easily accessible commercial products in order to obtain a methylglycine ester of Thalidomide.

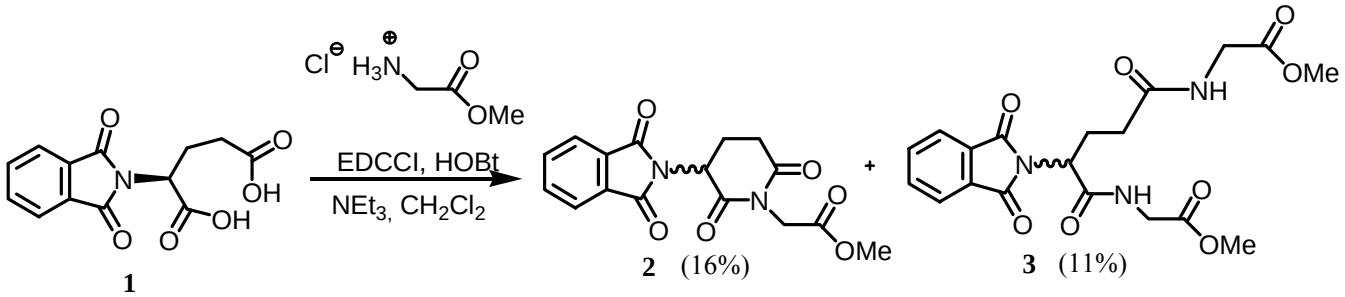


Thalidomide

Figure 1

RESULTS AND DISCUSSION

The first synthesis consisted in a condensation between *(S)*-phtaloylglutamic acid (**1**) and glycine methyl ester hydrochloride (Scheme 1) in the presence of triethylamine (1 mol).[10] This reaction led to the expected cyclic imide (**2**), under its racemic form. The loss of stereochemistry was of minor importance in this case because of the spontaneous racemization of Thalidomide at physiological pH. The second product (**3**) was also formed, resulting from a condensation between two molecules of glycine methyl ester and one mole of phtaloylglutamic acid.



Scheme 1

In order to improve the yield in compound (**2**), the reaction was realized using different amounts of triethylamine (Table 1). Surprisingly, the best results were obtained without addition of triethylamine, the reaction leading then to 16% of the desired cyclic imide and 15% of the side-product. It proved that it was not necessary to deplace hydrochloric acid from its glycine salt before the condensation. In the presence of the base, the total yield of the reaction tended to decrease, and with two equivalents of triethylamine and more, for one equivalent of *(S)*-phtaloylglutamic acid (**1**), compound (**3**) remained the major product.

Triethylamine (Eq)	0	1	2	3	5
Compound (**2**)	16%	16%	7%	7%	7,5%
Compound (**3**)	15%	11%	20%	21%	16%

Table 1

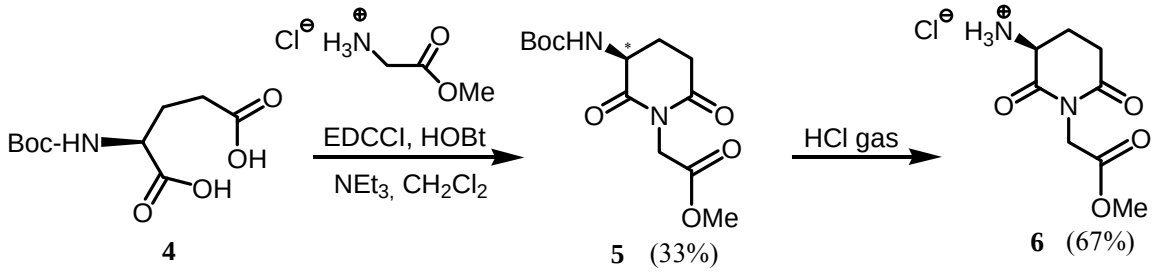
Cyclization reactions being generally optimized by concentrated reactional media, the same experiment was carried out with three different volumes of dichloromethane using 2 equivalents of triethylamine (Table 2). The ratio between **2** and **3** proved to be influenced by the dilution, but not in a classical way. The best ratio between **2** and **3** was obtained when the reaction was conducted in 60 mL of dichloromethane. Compound (**3**) became the main product with 30 mL of solvent.

Dichloromethane (mL)	15	30	60
Compound (**2**)	16%	7%	21%
Compound (**3**)	12%	20%	7.5%

Table 2

The lack of regioselectivity of this reaction did not allow to obtain the cyclic ester derivative (**2**) in good yields.

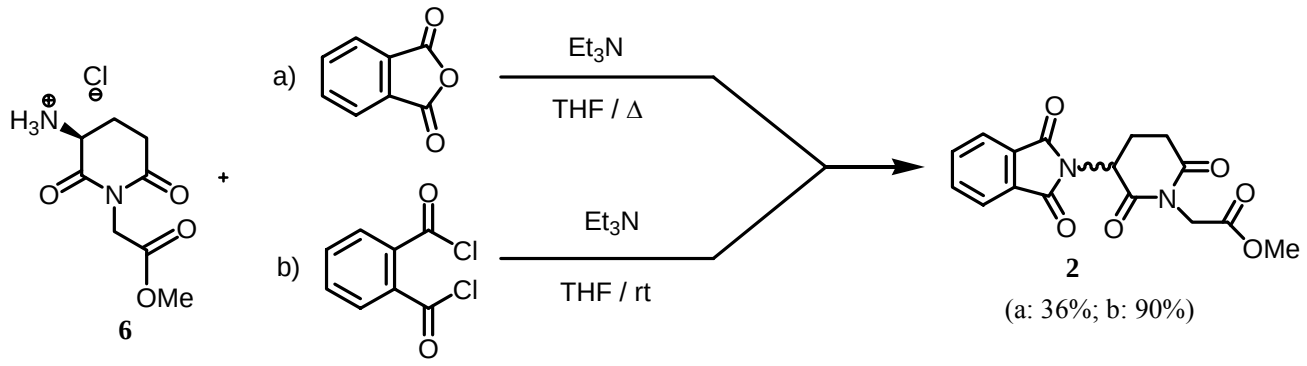
The second pathway consisted in the formation of the glutarimide ring followed by the synthesis of the phtalimide ring.[11] The starting material was then *(S)*-Boc-glutamic acid (**4**) (Scheme 2).



4 **5** (33%) **6** (67%)

Scheme 2

The heterocyclization reaction showed a better selectivity, leading to the formation of the single desired cyclic imide (**5**) in relatively low yield. The protective group was then removed by hydrochloric acid. The absolute configuration of the asymetric carbon bearing the amino function was kept in these two first steps. But, the condensation of compound (**6**) with phthalic anhydride or phthalic acid dichloride (Scheme 3) led to a racemic Thalidomide ester derivative with 90% yield in the last case (b).

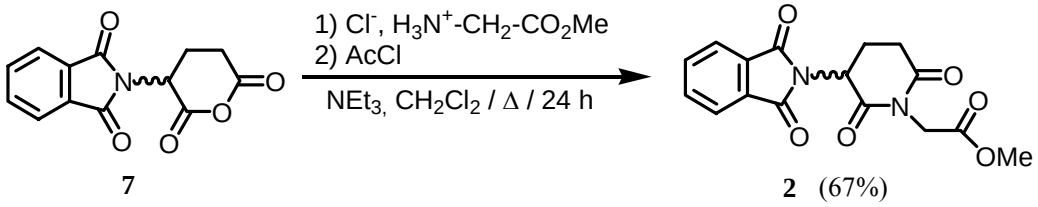


Scheme 3

Anyway, the total yield of this three steps synthesis was about 20% in the best case, the limiting step being the obtention of compound (**5**).

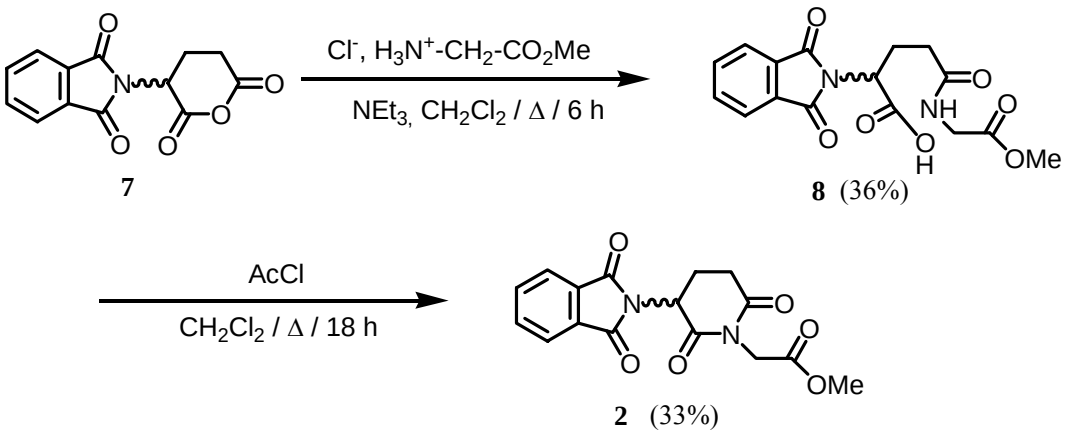
So, these two synthetic pathways showed low yields and led both to racemized compounds. The use of a commercially available racemic starting material was then considered in order to increase the yield and to realize a one step synthesis.

The synthesis of piperidinediones in good yields from corresponding pentanedioic anhydrides had been published.[12] A third synthesis from racemic phthaloylglutamic anhydride (**7**) was then studied. Introduction of a nitrogen atom instead of the oxygen atom of the oxine cycle was obtained in a one-pot synthesis using first glycine methyl ester and then acetyl chloride. Cyclic imide was obtained by this pathway in 67% yield (Scheme 4).



Scheme 4

In the absence of acid chloride, an intermediate (**8**) corresponding to a monoamidification of phthaloylglutamic acid was obtained. If compound (**8**) was refluxed for 18 h in dichloromethane in the presence of acetyl chloride, it led then to the imide (**2**) (Scheme 5). This proved that the assistance of the acyl group of acetyl chloride was necessary to obtain a cyclization.



Scheme 5

So, this last approach constituted the most efficient method to synthesize in one single step an ester derivative of Thalidomide from a commercially available starting material.

EXPERIMENTAL

TLC was carried out on SiO_2 (Kieselgel 60 F_{254}), and the spots were located with UV light except for compound (**4**) for which *o*-tolidine has been used as chromagen for the detection. *o*-Tolidine reagent was prepared by dissolution of 200 mg of *o*-tolidine in a mixture of 10 mL of hydrochloric acid (38 wt%) and 240 mL of ethanol. Columns chromatography were carried out on SiO_2 (Chromagel 60 ACC). Melting points were determined on a Kofler hot-plate melting point apparatus and are not corrected. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were taken on a Bruker Avance DMX spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an internal standard. MS spectra were obtained on a GC-MS system. A Agilent 6890 gas chromatograph was equipped with a non polar capillary column. The carrier gas was helium. The mass spectrometer (Hewlett Packard 5970 MSD) was operating in electron impact mode (70 eV) and directly interfaced with the gas chromatograph apparatus. The specific rotations were determined at 589 nm in 0.1 dm-cell with a Perkin-Elmer polarimeter (241-MC).

Preparation of compound (2) from (S)-Phthaloylglutamic acid (1)

To a stirred solution of *(S)*-Phtaloylglutamic acid (1.40 g, 5 mmol), glycine methyl ester hydrochloride (0.445 g, 5 mmol) and 1-hydroxybenzotriazole (1.50 g, 11 mmol) in dichloromethane (15, 30 or 60 mL, see Table 2) at 0°C, triethylamine (2.1 mL, 11 mmol) was eventually added, followed by the addition of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.11 g, 11 mmol). The reaction was allowed to warm up to rt, and stirred for 24 h. The solvent was evaporated under reduced pressure and the residue was chromatographed (cyclohexane – ethyl acetate, 50/50, v/v).

Methyl 2-[2,6-dioxo-3-(1,3-dioxoisoindolin-2-yl)piperidino]acetate (**2**): mp 114-116°C (cyclohexane); IR

(nujol) ν_{max} 3100, 2850, 1770, 1730, 1700 cm^{-1}; ^{1}H-NMR (300 MHz, CDCl3) δ 2.02 (1H, m), 2.70-2.92 (3H, m), 3.67 (3H, s), 4.50 (2H, s), 5.05 (1H, m), 7.62-7.72 (4H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 22.26, 32.03, 41.66, 48.33, 50.19, 52.93, 124.18, 124.39, 131.89, 132.10, 134.87, 135.14, 167.64, 168.30, 168.68, 170.88; MS m/z (relative intensity) 330 ($M^{+\cdot}$, 6.2), 271 (16.9), 243 (27.7), 215 (16.9), 183 (76.9), 173 (100); *Anal.* Calcd for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.24; N, 8.48. Found: C, 57.99; H, 4.32; N, 8.47.

Dimethyl [2-(1,3-dioxoisoindolin-2-yl)pentanediamido]diacetate (**3**): mp 30-32°C (cyclohexane); IR (nujol) ν_{max} 3550, 3100, 2900, 1770, 1720 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.30-2.70 (4H, m), 3.62 (3H, s), 3.65 (3H, s), 3.92 (4H, m), 4.80 (1H, m), 7.66 (4H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 25.37, 33.01, 41.59, 41.72, 52.92, 54.15, 124.01, 131.99, 134.68, 168.46, 169.63, 171.13, 172.98; MS m/z (relative intensity) 419 ($M^{+\cdot}$, 7.7), 388 (2.3), 331 (9.2), 303 (16.9), 289 (20), 186 (89.2), 156 (100); *Anal.* Calcd for $C_{19}H_{21}N_3O_8$: C, 54.42; H, 5.01; N, 10.02. Found: C, 54.36; H, 5.14; N, 9.91.

Preparation of compound (2) from *(S)*- Boc-glutamic acid (4)

Methyl 2-[(S)-3-*tert*-butoxycarbonylamino-2,6-dioxopiperidin-1-yl]acetate (**5**): To a stirred solution of *(S)*-Boc-glutamic acid (1.24 g, 5 mmol), glycine methyl ester hydrochloride (0.445 g, 5 mmol) and 1-hydroxybenzotriazole (1.50 g, 11 mmol) in dichloromethane (30 mL) at 0°C was added triethylamine (2.1 mL, 11 mmol) followed by the addition of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.11 g, 11 mmol). The reaction was allowed to warm up to rt, and stirred for 24 h. The solvent was evaporated under reduced pressure and the residue was chromatographed (cyclohexane – ethyl acetate, 50:50, v/v): mp 129-131°C (cyclohexane); IR (nujol) ν_{max} 3400, 2900, 1770, 1750, 1695 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.93 (1H, m), 2.48-2.96 (3H, m), 3.73 (3H, s), 4.38-4.80 (3H, m), 5.41 (1H, br s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 25.09, 28.67, 31.89, 41.56, 52.72, 52.93, 80.85, 155.90, 168.50, 171.25, 172.01; MS m/z (relative intensity) 300 ($M^{+\cdot}$, 0.1), 243 (8.9), 227 (17.4), 213 (19.4), 201 (32.2), 57 (100); $[\alpha]_D^{20}$ -23.6 (c=0.01, $CHCl_3$); *Anal.* Calcd for $C_{13}H_{20}N_2O_6$: C, 52.00; H, 6.67; N, 9.33. Found: C, 51.71; H, 6.86; N, 9.22.

Methyl 2-[(S)-3-amino-2,6-dioxopiperidin-1-yl]acetate hydrochloride (**6**): Compound (**5**) (450 mg, 1.50 mmol) was dissolved in 15 mL of dichloromethane. HCl gas was bubbled into the solution for 1 h and the mixture was stirred for 8 h. The precipitate was filtered and washed with dichloromethane to afford after drying 200 mg (67%) of the product: mp 144-146°C (ether); IR (nujol) ν_{max} 3400, 3200, 1750, 1720 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.05 (1H, m), 2.30 (1H, m), 2.79-2.94 (2H, m), 3.65 (3H, s), 4.39-4.45 (3H, m), 8.84 (2H, br s); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 21.63, 30.41, 41.09, 49.80, 52.67, 168.41, 169.79, 171.14; MS m/z (relative intensity) 200 ($M^{+\cdot}$, 1.8), 169 (10), 141 (7.7), 84 (100), 56 (67.7); $[\alpha]_D^{20}$ -50.0 (c=0.01, DMSO); *Anal.* Calcd for $C_8H_{12}N_2O_4$: C, 40.59; H, 5.52; N, 11.89. Found: C, 39.75; H, 5.59; N, 11.68.

Methyl 2-(2,6-dioxo-3-(1,3-dioxoisoindolin-2-yl)piperidin-1-yl)acetate **(2)**:

Pathway a: Compound (**6**) (200 mg, 0.85 mmol) was dissolved in 20 mL of anhydrous tetrahydrofuran and 4Å molecular (500 mg) sieves were added. Triethylamine (1.45 mL, 6.8 mmol) and *o*-phthaloyl dichloride (0.145 mL, 1 mmol) were successively added. The mixture was stirred at rt for 17 h. The solvent was evaporated under reduced pressure and the residue was chromatographed (cyclohexane – ethyl acetate, 50:50, v/v) to afford 250 mg (89 %) of the product.

Pathway b: Compound (**6**) (200 mg, 0.85 mmol) was dissolved in 20 mL of anhydrous tetrahydrofuran and 4Å molecular sieves (500 mg) were added. Triethylamine (1.45 mL, 6.8 mmol) and phthalic anhydride (157 mg, 1 mmol) were successively added. The mixture was refluxed for 9 h. The solvent was evaporated under reduced pressure and the residue was chromatographed (cyclohexane – ethyl acetate, 50:50, v/v) to afford 250 mg (33 %) of the product.

Preparation of compound (2) from Phthaloylglutamic anhydride (7)

To a solution of glycine methyl ester hydrochloride (930 mg, 7.40 mmol) in 10 mL of dichloromethane at 0°C were added phthaloyl glutamic anhydride (2 g, 7.70 mmol) and triethylamine (1.4 mL, 6.60 mmol). The solution was stirred for 5 min at 0°C. Acetyl chloride (13 mL, 183 mmol) was then added, and the solution was refluxed for 24 h. The solvent was evaporated under reduced pressure and the residue was chromatographed (cyclohexane – ethyl acetate, 50:50, v/v) to afford 1.64 g (67 %) of the product.

Preparation of compound (2) *via* formation of compound (8)

4-methoxycarbonylmethylcarbamoyl-2-(1,3-dioxoisoindolin-2-yl)butanoic acid **(8):** To a solution of glycine methyl ester hydrochloride (930 mg, 7.40 mmol) in 10 mL of dichloromethane at 0°C was added phthaloyl glutamic anhydride (2 g, 7.70 mmol) and triethylamine (1.4 mL, 6.60 mmol). The solution was then refluxed for 24 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in 10 mL of dichloromethane. After filtration, the filtrate was then evaporated under reduced pressure. The residue was dissolved in 50 mL of 37 % hydrochloric acid. The aqueous acidic solution was shaked with 5 times 50 mL dichloromethane in a separatory funnel. The organic layer was separated and dried over magnesium sulfate. After filtration, the solvent was evaporated under reduced pressure and the residue was chromatographed (tetrahydrofuran – ethyl acetate, 50:50, v/v) to afford 960 mg (36%) of the product: mp 157-159°C (toluene); IR (nujol) ν_{max} 3500, 3100, 2900, 1770, 1740 cm^{-1};^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.04-2.35 (4H, m), 3.45 (3H, s), 3.57 (2H, s), 4.61 (1H, m), 7.75 (4H, m), 8.18 (s, 1H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 21.34, 31.23, 41.24, 49.82, 52.50, 123.83, 131.53, 135.30, 167.40, 168.33, 169.59, 171.61; *Anal.* Calcd for $C_{16}H_{16}N_2O_7$: C, 55.16; H, 4.64; N, 8.04. Found: C, 55.04; H, 4.67; N, 7.93.

Methyl 2-(2,6-dioxo-3-(1,3-dioxoisoindolin-2-yl)piperidin-1-yl)acetate **(2)**: To a solution of compound **(8)** (317 mg, 0.91 mmol) in 1.2 mL of dichloromethane was added acetyl chloride (1.5 mL, 21 mmol). The mixture was then refluxed for 18 h. The solvent was evaporated under reduced pressure and the

residue was chromatographed (cyclohexane – ethyl acetate, 50:50, v/v) to afford 100 mg (33 %) of the product.

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