A SIMPLE, MICROWAVE-ASSISTED METHOD FOR SYNTHESIS OF THALIDOMIDE

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SUMMARY

Objectives: To investigate the conditions for the synthesis of thalidomide in two-step with the assistance of microwave irradiation resulted in high overall yield. Method: Preparation of thalidomide which comprises reacting anhydride phthalic with L-glutamic acid to afford N-phthaloyl-DL-glutamic acid, which is further subjected to cyclization with ammonia donor sources (urea, ammonium acetate, thiourea) in presence of 4-dimethyl-aminopyridine and diphenyl either to give thalidomide. Results: Investigating the conditions of the reaction including temperature, duration and mode of reaction; ratio of reactive agents of preparation of thalidomide. From these results, we found out the conditions to synthesize this compound. Conclusion: An improved synthesis for thalidomide was established. It produced a total yield of 72% over two steps.

** Keywords: Thalidomide; Phthalic acid; L-glutamic acid; Synthesis.*

INTRODUCTION

Thalidomide (I)/(N-phthalimidogluatarimide)/ was first marketed in West Germany by Chemie Grunelthal GmbH as a clinically effective and extremely safe non-barbiturate sedative-hypnotic in 1957 [1]. This drug was used therapeutically as sedative-hypnotic from 1958 to 1961. It became a popular drug in Europe, Japan, and Canada with a variety of trade names: Contergan®, Isomin®, and Distaval®, for example. In 1961, W. Lenz [2] and W.G. McBride [3] realized the unexpected potent teratogenicity of thalidomide. The teratogenic side effects, leading to birth defects such as limb reduction, produced one of the most notorious medical disasters in modern medical history and thalidomide was consequently withdrawn from the market in 1962.

However, the unique and broad physiological effects of thalidomide have gradually revealed in succession with the discovery of its effectiveness toward other diseases as leprosy, rheumatoid arthritis, neoplastic diseases, HIV/AIDS, multiple myeloma, mesothelioma, Crohn's diseases, cancer-related to pathologic angiogenesis,

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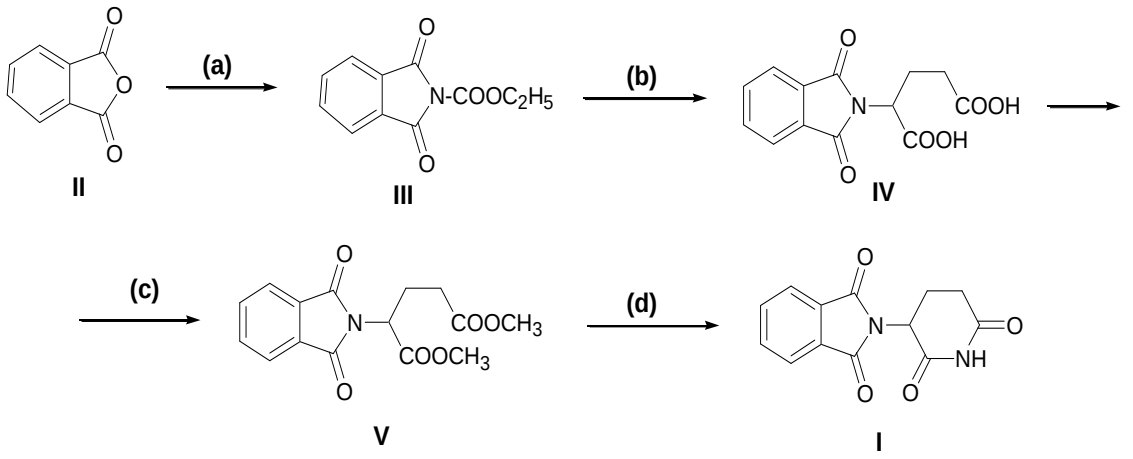
and other diseases. Thus, in 1998, Celgene received thalidomide FDA approval to use thalidomide (thalomid) for the treatment of ENL. More recently, thalidomide has been connected with the treatment of several diseases such as leprosy [4], AIDS [5], Crohn's diseases [6], rheumatoid arthritis [7], cancer-related to pathologic angiogenesis [8] and it is still under study for other diseases [9].

However, due to its catastrophic effect on fetal malformation, it was banned in the early sixties. In recent years, the concerns about this drug has been on the increase for the treatment of the above-mentioned diseases attracting interest in the development of new improved synthetic approaches of thalidomide and its derivatives.

A number of publications about the synthesis of thalidomide were reported from starting pair of materials or different starting materials such as: anhydride phthalic and L-glutamic acid [10]; phthalic anhydride and L-glutamine [11]; phthalic anhydride and 2.6-dioxo-3-amino-pyridine or its derivatives [12]. Although these synthetic procedures seem to be straight forward transformations, they suffer from several drawbacks on the large-scale preparation: (1) Use of costly starting reagents/materials in the steps involved the preparation; (2) Reactions carried out involving a high melting temperature requiring multiple recrystallizations [1] or purified by column chromatography on silica gel [11a]; (3) Using toxic solvents/materials; (4) Procedure have lot of steps [1,11a]; (5) Low overall yields [10b]. Usually, in finishing step - the conditions employed for cyclization of the glutarimide-ring including the condensation of Na/liquid or gas ammonia at high pressure [10b]; the reaction urea/thiourea in melting mixture at high temperature [1], the cyclization of the amide with CDI/4-DMPA or CDT/4-DMAP [11c]. These conditions can often cause low yields, longer reaction times and byproduct formation. That is, none of these procedures is practical in terms of industrial scale-up operations.

Several groups have reported the synthesis of thalidomide (I) from phthalic anhydride (II) via three or four steps with relatively low overall yields [1, 10b] whereby *N*-carbethoxylation of II produces *N*-carbethoxy-phthalimide (III), respectively. Conversion of III to *N*-phthaloyl-*DL*-glutamic acid (IV) with *L*-glutamic acid and sodium carbonate in water and then esterification of IV with methanol and thionyl chloride in reflux conditions give *N*-phthaloyl *L*-glutamic acid dimethyl diester (V). Finally, the compound V was treated with sodium amide (prepared in situ from metal and ammonia in the presence of iron (III) nitrate in liquid ammonia and ammonium chloride to afford white solid, which was purified by column chromatography to give thalidomide, with low overall yields (19% from III) (*Scheme 1*) [10b].



Scheme 1: Four-step synthesis of thalidomide (I) from phthalic anhydride (II) and e4dsxz*L*-glutamic acid [10b].

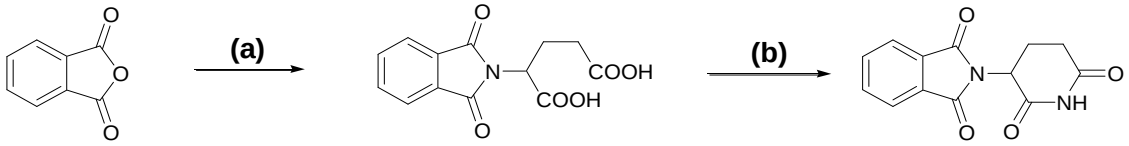
(Reagents and conditions: (a) i, NH_3/high temperature and pressure; ii, $ClCOOC_2H_5$; (b) L-glutamic acid/Na_2CO_3/0°C/5 mins + RT/40 mins, 60%; (c) Methanol/SO_2Cl_2 /reflux/6h/purified on column chromatography, 71%; (d) Na/liquid NH_3/Fe_3NO_3, 45%)

MATERIALS AND METHODS

All of the commercially available reagents and solvents were used without further purification. The ^{1}H-NMR and ^{13}C-NMR spectra were measured in $CDCl_3$ on Bruker-AV500 spectrometer; the chemical shifts were reported in ppm relative to TMS. The IR spectra were recorded in the solid state as KBr dispersion using a GX-Perkin Elmer spectrophotometer (USA). The mass spectra (70 *eV*) were recorded on AutoSpec Premier Spectrometer. The melting points were measured on Stuart SMP-10 apparatus. Analytical thin layer chromatography (TLC) was carried out on Merck pre-coated aluminum silica gel sheets (Kieselgel 60F-254). Sineo Microware Chemistry Technology UWare-1000 (China).

RESULTS AND DISCUSSION

In this report, the pair of starting materials phthalic anhydride (II) and *L*-glutamic acid was chosen for the preparation of intermediate *N*-phthaloyl-*DL*-glutamic acid (IV), because of high cost. The IV was prepared by treatment of II with *L*-glutamic acid in pyridine at 115°C for 15 mins by microwave irradiation and then the reaction mixture was added to water and adjusted pH to 1.2 with 6N HCl solution. The product as white solid was separated, filtered and washed with cooled water to afford IV in 90%. This material requires no further purification in the next step. The mixture of IV, thiourea (as a source of ammonia) and diphenyl ether in presence of 4-DMAP was heated by microwave irradiation at 178°C for 12 minutes after work-up receive thalidomide (I) in 80% *(scheme 2)*.



II IV I

Scheme 2: Two-step synthesis of thalidomide from phthalic anhydride and L-glutamic acid.

(Reagents and conditions: (*a) L-glutamic acid/pyridine/115°C/15 mins, 89% (b) Thiourea/4-DMAP/diphenyl ether/178°C/15 mins, 81%)*

- Synthesis of N-phthaloyl-DL-glutamic acid (IV): Compound IV was prepared from phthalic anhydride (II) and *L*-glutamic acid. In this reaction, the mixture of II, *L*-glutamic acid and pyridine was stirred and heated to 115°C by microwave apparatus. This method bypassed the carbethoxylation of II to afford III (*scheme 1*, step a), thus eliminating the need for preparation of *N*-carbethoxy-phthalimide (III). This change reduced one step of the procedure. In addition, the parameters of procedure as solvent type *(table 1)*; the reaction temperature *(table 2)*; the water volume using in isolation of N-phthaloyl-DL-glutamic acid (IV) from reaction mixture *(table 3)*; the molar ratio between reactants *(table 4)*; the pyridine volume used in reaction *(table 5)* was optimized.

Table 1: Effect of reaction solvent on the yield of N-phthaloyl-DL-glutamic acid (IV).

No.	Solvent	Temperature (°C)	N-phthaloyl-DL-glutamic acid (IV)		
			Weight (g)	Melting point (°C)	Yield (%)
1	Pyridine	115	11.66	191 - 193	84.21
2	DMF	153	10.32	191 - 192	74.54
3	Dioxane	101	6.72	191 - 193	48.54
4	4 mL dioxane + 1 mL DMF	101	6.94	192 - 193	50.10
5	Acetonitrile	81	9.63	192 - 193	69.55

The optimal solvent was pyridine (N°1).

Table 2: Effect of reaction temperature on the yield of IV.

No.	Temperature (°C)	N-phthaloyl-DL-glutamic acid (IV)		
		Weight (g)	Melting point (°C)	Yield (%)
1	115	11.66	191 - 193	84.21
2	100 - 102	11.13	191 - 192	80.38
3	80 - 82	10.30	191 - 192	74.34

The reaction temperature gives the best yield of IV was 115°C (No.1).

Table 3: Effect of reaction water volume on the yield of IV.

No.	Water volume (mL)	N-phthaloyl-DL-glutamic acid (IV)		
		Weight (g)	Melting point (^{0}C)	Yield (%)
1	45	11.66	192 - 193	84.21
2	60	12.04	191 - 193	86.92
3	75	11.80	191 - 192	85.17

The optimal water volume was 60 mL (No.2).

Table 4: Effect of molar ratio between anhydride phthalic and L-glutamic acid on the yield of N-phthaloyl-DL-glutamic acid (IV).

No.	Molar ratio of anhydride phthalic and L-glutamic acid	N-phthaloyl-DL-glutamic acid (IV)		
		Weight (g)	Melting point (^{0}C)	Yield (%)
1	1:0.9	11.24	192 - 193	81.17
2	1:1	12.05	191 - 193	87.03
3	0.9:1	11.12	191 - 192	80.29

The result found that using molar ratio of anhydride phthalic:L-glutamic acid was 1:1, which got the highest yield *(*No.2*).*

Table 5: Effect of solvent volume on the yield of N-phthaloyl-DL-glutamic acid (IV).

No.	Pyridine volume (mL)	N-phthaloyl-DL-glutamic acid (IV)		
		Weight (g)	Melting point (^{0}C)	Yield (%)
1	3	9.86	191 - 193	71.18
2	4	10.88	192 - 193	78.56
3	5	12.32	191 - 193	88.92
4	6	11.38	191 - 192	82.17
5	7	11.32	192 - 193	81.73

The optimal pyridine volume with the highest yield was 5 mL per 0.05 mole anhydride phthalic (No.3).

- Synthesis of thalidomide (I): Compound I was synthesized in one step from IV by heating a mixture of IV, thiourea (ammonia donor source), 4-DMAP as catalytic, diphenyl either in microwave apparatus instead of two steps were esterification of IV with methanol/SO_2Cl_2 to afford ester V and then the formation the glutarimide-ring from V by using the $NaNH_2$/liq.NH_3/ $Fe(NO_3)_3$. The parameters of this step were also optimized for cyclization of glutarimide-ring, which resulted in the reaction temperature (178^{o}C) and a

shorter reaction time (15 mins). Those parameters consist of the ammonia donor source; the power of microwave and the reaction temperature; the molar ratio between thiourea:compound IV; the solvent type which used in reaction; and the diphenyl ether volume used in reaction *(table 6 - 10).* Finally, the method of separation and purification of I was also investigated. As a result, there was no need to column chromatography for the purification of thalidomide.

Table 6: Effect of ammonia donor source on the yield of thalidomide (I).

No.	Ammonia donor source	Thalidomide		
		Weight (g)	Melting point (^{0}C)	Yield (%)
1	Urea	3.21	269 - 271	62.23
2	Thiourea	3.75	269 - 270	72.65
3	Ammonium acetate	3.08	269 - 271	59.61
4	Ammonium chloride	1.56	270 - 271	30.31

The result found that using thiourea as ammonia donor source got the highest yield of I (No.2).

Table 7: Effect of reaction solvent on the yield of thalidomide.

No.	Solvent	Temperature (^{o}C)	Thalidomide		
			Weight (g)	Melting point (^{0}C)	Yield (%)
1	DMF	153	2.02	269 - 270	39.19
2	DMA	165	2.08	269 - 270	40.23
3	Ph_2O	180	3.74	269 - 271	72.55

The optimal solvent was diphenyl ether (No.3).

Table 8: Effect of reaction temperature on the yield of thalidomide.

No.	Temperature (°C)	Thalidomide		
		Weight (g)	Melting point (^{0}C)	Yield (%)
1	160	3.58	269 - 270	69.43
2	170	3.75	269 - 271	72.65
3	175	3.93	269 - 271	76.21
4	180	3.95	269 - 271	76.55
5	185	3.89	270 - 271	75.36
6	200	3.62	269 - 270	70.17

The reaction temperature gives the best yield of I was 178°C (between 175 and 180°C) (No.4).

Table 9: Effect of molar ratio between thiourea and compound IV on the yield of 1.

No.	Molar ratio of thiourea:compound IV	Thalidomide (1)		
		Weight (g)	Melting point (^{0}C)	Yield (%)
1	1:1	3.62	269 - 270	70.06
2	2:1	3.75	269 - 270	72.61
3	3:1	4.06	269 - 271	78.69
4	4:1	3.93	270 - 271	76.12
5	5:1	3.92	269 - 270	75.91

The result found that using molar ratio of thiourea:compound IV was 3:1 which got the highest yield (No.3).

Table 10: Effect of reaction solvent volume on the yield of thalidomide.

No.	Ph_2O volume (mL)	Thalidomide		
		Weight (g)	Melting point (^{0}C)	Yield (%)
1	3	3.40	269 - 271	65.82
2	4	3.75	269 - 270	72.58
3	5	4.17	269 - 271	80.84
4	6	4.05	270 - 271	78.56
5	7	4.00	269 - 270	77.55

The optimal diphenyl ether volume with the highest yield was 5 mL per 0.02 mole compound IV (No.3).

- Synthesis of *N*-phthaloyl-*DL*-glutamic acid (IV): A mixture of phthalic anhydride II (11.2 g, 0.075 mole), *L*-glutamic (11.0 g, 0.075 mole) and pyridine (75 mL) in a round-bottom flask was subjected to microwave irradiation (100 W, 115^{o}C, 15 mins) with stirring. After the reaction was finished (15 mins), the round-bottom flask was then removed from the microwave apparatus. The reaction mass was cooled to 75^{o}C and ice-cold water (90 mL) was added with stirring, the reaction mixture was adjusted to pH 1.2 with 6N HCl and stirring at 10 - 15^{o}C for 2h. The white solid was separated, filtered, and washed with cool water (3 x 10 mL). The obtained product was dried under vacuum to afford *N*-phthaloyl-*DL*-glutamic acid (IV) (18.67g, 89.34%), mp: 191 - 193ºC R_f = 0.41 (benzene:dioxane:formic acid = 75:20:5).

- IR (KBr) u_{max} (cm-1): 3447.69 (O-H) 3057.72 (CH,); 2923.11 (CH, CH_2); 1716.05 (C=O). MS: m/z 276 [M-H]. ^{1}H-NMR (DMSO) δ (ppm): 12.65 (s, 2H, COOH); 7.88 - 7.90 (m, 4H: C5-H, C6-H, C7-H, C8-H); 4.79 - 4.82 (m, 1H, C10-H); 2.25 - 2.51 (m, 4H, C11-H2 and C11-H2). ^{13}C-NMR (DMSO) δ (ppm): 173.70 (C13); 170.31 (C14); 167.44 (C1, C3); 134.75 (C6 and C7); 131.28 (C4 and C9); 123.36 (C5 and C8); 51.08 (C10); 30.36 (C12); 23.69 (C11).

- Synthesis of thalidomide (I): In a round-bottom flask, the mixture of *N*-phthaloyl-*DL*-glutamic acid (IV) (16.7 g, 0.06 mole), thiourea (13.7 g, 0.18 mole) and 0.015 g 4-dimethylamino-pyridine, diphenyl ether (15 mL) was added. The above reaction mass was subjected to the microwave apparatus (100 W, 178°C, 15 mins) with stirring. After the reaction was terminated (15 mins), the round-bottom flask was then removed from the microwave apparatus. The reaction mass was cooled to 100°C and toluene (45 mL) was added, stirring for 20 mins, the reaction was cooled to 5 - 10°C for 1h. The white solid was separated, filtered, and washed with cool water (3 x 10 mL) received a solid product. To this product, methanol (45 mL) was added, stirring and heating to reflux for 20 mins, distilling out solvent 1/2 volume, cooling to 10 - 15°C for 2h, filtering to give a crude product. This process was repeated two times to give thalidomide. Recrystallization of raw thalidomide from dioxane-acetone. The obtained product was air-dried and then dried under vacuum (60°C, < 1 mmHg) to afford thalidomide (12.62 g, 81.25%), mp: 270 - 272ºC; R_f = 0,5 (benzene:dioxane: formic acid = 75:20:5).

- IR (KBr) υ_{max} (cm-1): 3204.53 (N-H); 3097.99 (CH) and 2924.13 (CH_2); 1776.46 (C=O, C1, C3); 1697.16 (C=O, C13 and C14). MS: m/z 257 [M-H]. ^{1}H-NMR (DMSO) δ (ppm): 11.12 (s, 1H, NH); 7.88 - 7.94 (m, 4H, C5-H, C6-H, C7-H, C8-H); 5.14 - 5.20 (1H, C10-H, J = 13.0 Hz and J = 5.5 Hz); 2.86 - 2.94 (m, 1H, C12-Ha); 2.05 - 2.10 (m, 2H, C11-H2); 2.05 - 2.10 (m, 1H, C12-Hb). ^{13}C-NMR (DMSO) δ (ppm): 171.72 (C13); 169.81 (C14); 167.13 (C1 and C3); 134.85 (C6 and C7); 131.21 (C4 and C9); 123.39 (C5 and C8); 48.98 (C10); 30.92 (C12); 21.97 (C11).

CONCLUSION

An improved synthesis for thalidomide (I) has been established *(scheme 2).* It produced a total yield of 72% over two steps (compared to overall yields of 45 - 58% in four steps). The synthesis of IV from II was successfully accomplished in one step reaction. The subsequent conversion of IV to I was carried out under milder reaction conditions without using hazardous solvents. Raw materials and reagents used in our procedure are economical and commercial. Each reaction step was optimized to reduce or eliminate the use of toxic reagents and solvents. Total preparation time was significantly reduced compared to those methods described previously. Our results suggested that this method is economically advantageous over the earlier reported approaches owing to its high yields and the use of less expensive raw materials. These advantages facilitate the efficient, cost-effective and industrially convenient production of thalidomide.

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