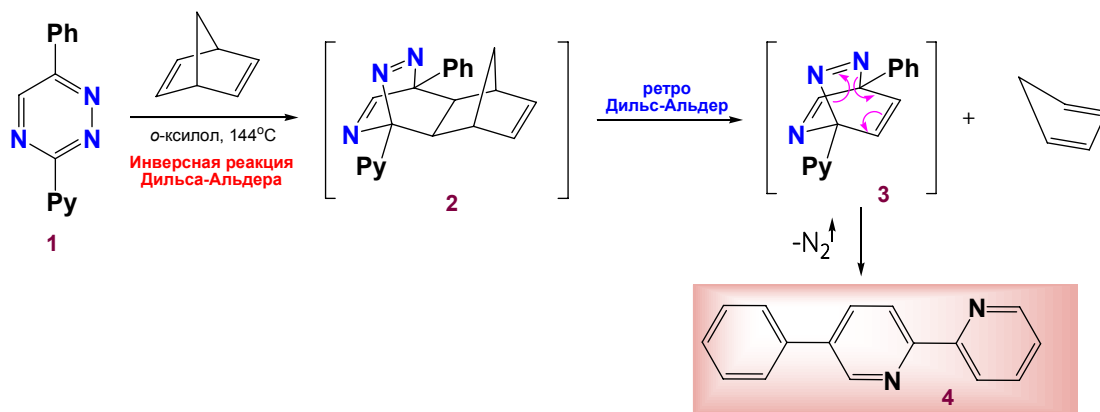
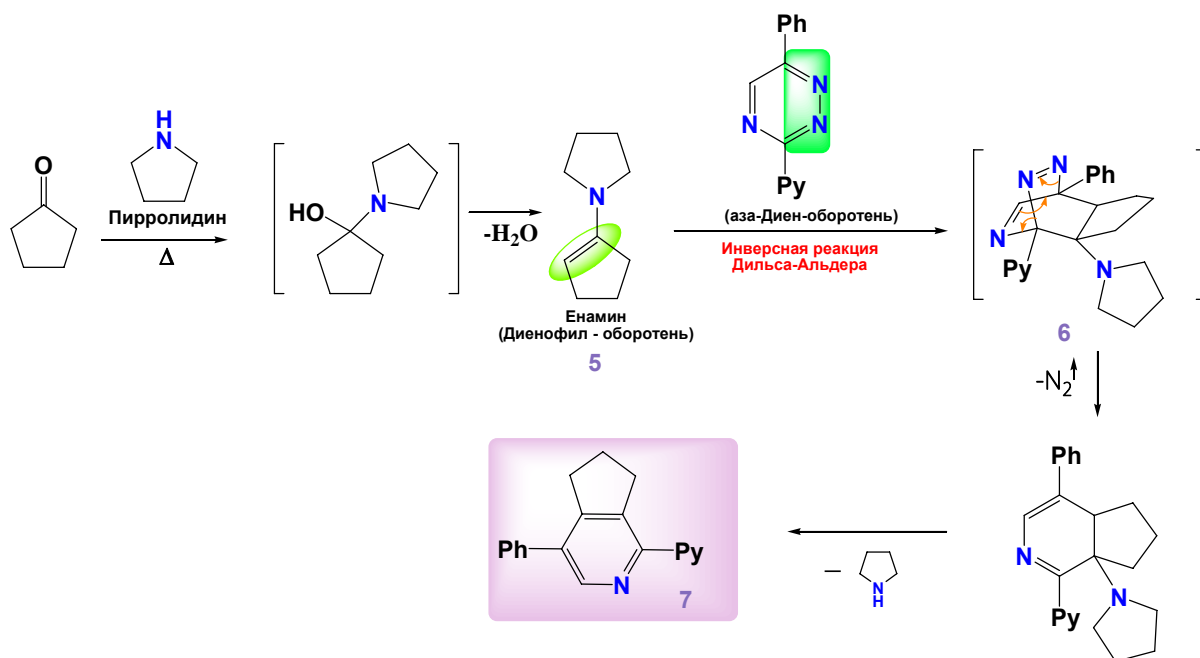


Задача 1

Преобразование дизамещенных 1,2,4-триазинов (**1**) в арилбипиридилы (**4**) осуществляется в результате протекания реакции циклоприсоединения по типу *аза-Дильса-Альдера*. В результате взаимодействия триазинового кольца субстрата (*диеновый компонент*) с напряженным диенофилом – 2,5-норборнадиеном, происходит первоначальное образование циклоаддукта (**2**), дальнейшее нагревание которого ведет к продуктам обратной реакции (*ретро-Дильса-Альдера*), но уже другого строения. Интермедиат процесса – триазабициклооктадиен (**3**) достаточно легко «отбрасывает» молекулу азота, превращаясь в ароматический пиридиновый цикл конечного продукта (**4**).



Второй процесс также представляет собой пример реакции *аза-Дильса-Альдера* с «обращенными» электронными требованиями (т.е. *диеном* выступает фрагмент, насыщенный электроноакцепторными группами, а *диенофилом* – олефин, имеющий явный электронодонорный остаток, в данном случае енамин (**5**), образующийся при взаимодействии циклопентанона с пирролидином). В этом случае, по-видимому, более вероятной, является первоначальное *деазотирование* в циклоаддукте (**6**), а уже затем потеря вторичного амина, ведущая к ароматизации конечного продукта (**7**).

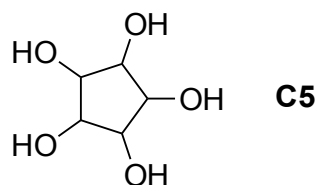


Задача составлена по мотивам оригинальной статьи: V.N. Kozhevnikov, D.N. Kozhevnikov, O.V. Shabunina, V.L. Rusinov and O.N. Chupakhin An efficient route to 5-(hetero)aryl-2,4'- and 2,2'-bipyridines through readily available 3-pyridyl-1,2,4-triazines *Tetrahedron Letters* (2005), v. 46, p. 1791–1793.

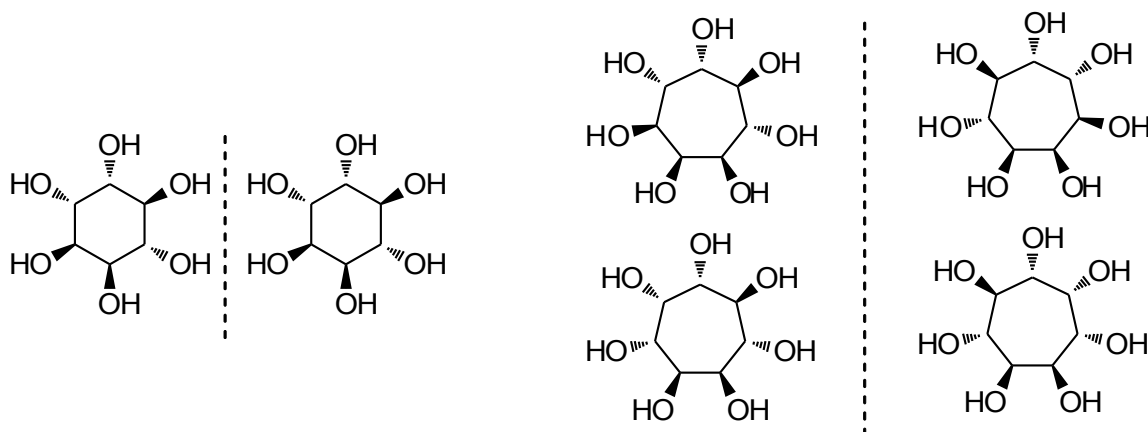
Подробнее об инверсных реакциях *аза-Дильса-Альдера* 1,2,4-триазинов можно прочитать в статьях D.L. Boger, J.S. Panek, and M.M. Meierlb (*J. Org. Chem.*, 1981, 46, 2179-2182; *J. Org. Chem.*, 1982, 47, 895-897.)

Задача 2.

Первая часть задачи самая простая, надо всего лишь нарисовать те изомеры, которые не обладают плоскостью зеркального отражения.

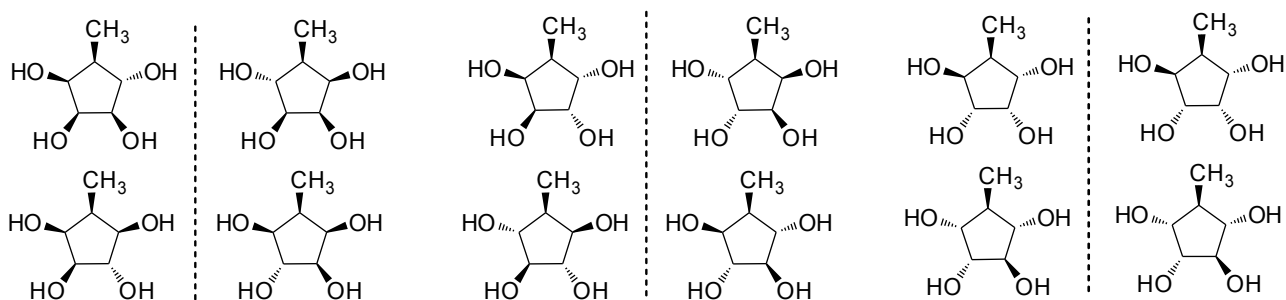


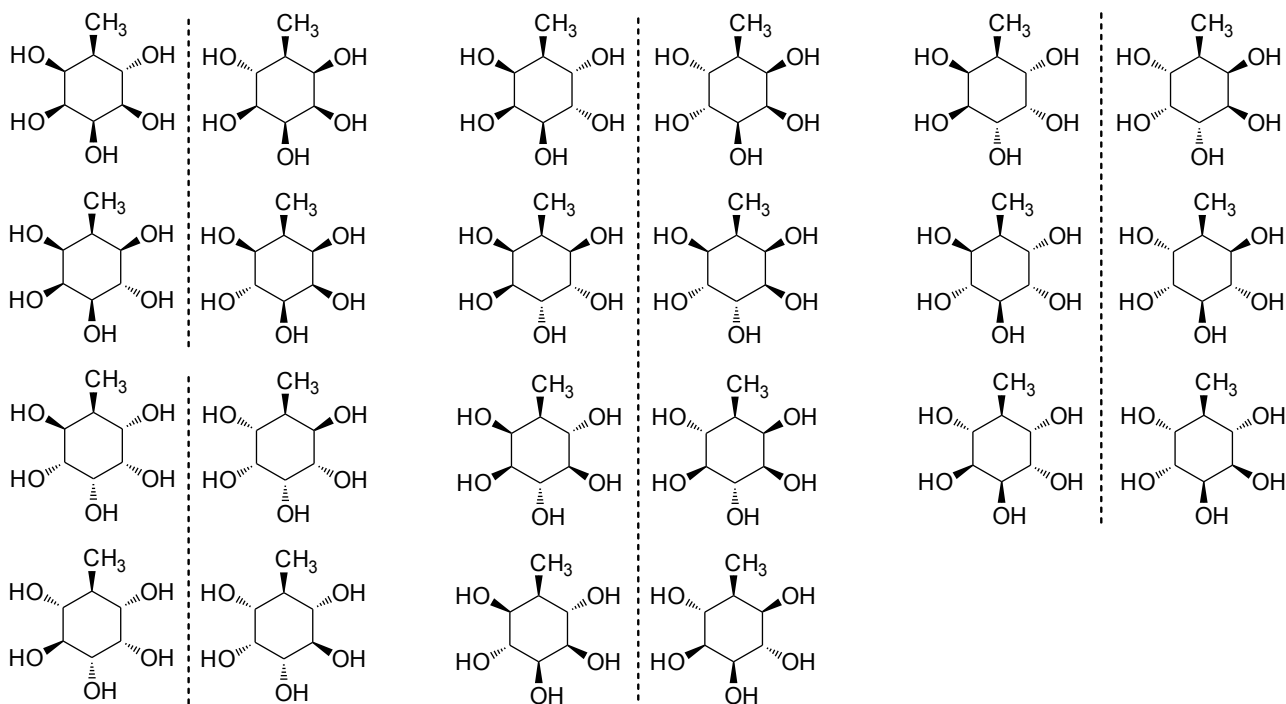
Это соединение, назовем его **C5**, оптическую активность не проявляет, т.к. при любой взаимной ориентации -ОН группы в пространстве можно найти плоскость зеркального отражения поэтому оптических изомеров у нее не будет.



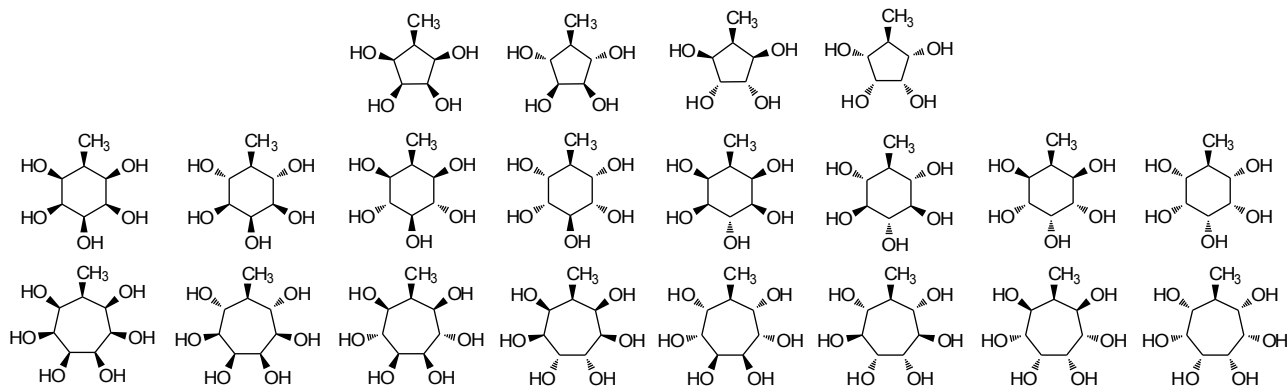
В структурах **C6** и **C7** -ОН группы возможно расположить таким образом чтобы исключить плоскости зеркального отражения. Таких оптических изомеров будет 2 для **C6** и 4- для **C7**.

Перейдем ко второй части задания. С первого взгляда, кажется, что самое простое нарисовать все асимметричные изомеры и посчитать их:





Но как вы поняли, это довольно скучно и долго! Давайте просто посчитаем сколько изомеров будет обладать плоскостью зеркального отражения и вычтем их количество из общего числа изомеров. Вот они на рисунке:



Всего число разных изомеров для каждого соединения будет равно $2^{n/2}$, т.е. для **C5-Me=16**, **C6-Me=32**, **C7-Me=64**. Соответственно количество оптических изомеров будет равно **C5-Me=12**, **C6-Me=26**, **C7-Me=58**.

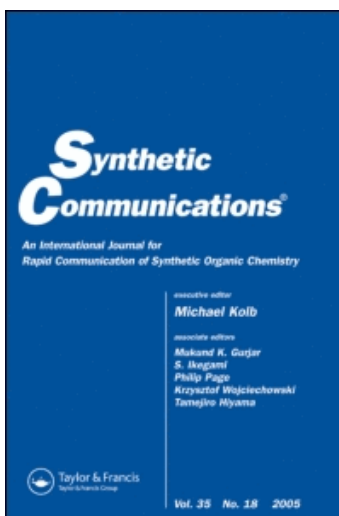
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Synthetic Communications

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A Synthesis of Racemic Thalidomide

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Online publication date: 15 April 2003

To cite this Article Chang, Meng-Yang, Chen, Shui-Tein and Chang, Nein-Chen (2003) 'A Synthesis of Racemic Thalidomide', *Synthetic Communications*, 33: 8, 1375 – 1382

To link to this Article: DOI: 10.1081/SCC-120018698

URL: <http://dx.doi.org/10.1081/SCC-120018698>

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SYNTHETIC COMMUNICATIONS®

Vol. 33, No. 8, pp. 1375–1382, 2003

A Synthesis of Racemic Thalidomide

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ABSTRACT

A synthesis of racemic thalidomide is described using formal [3+3] cycloaddition strategy as the key step. The total yield of thalidomide was 30% in three steps from ester **2**.

Key Words: Thalidomide; Formal [3+3] cycloaddition; Glutaramides; α -sulfonylacetamide; Ester.

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DOI: 10.1081/SCC-120018698
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INTRODUCTION

Glutarimides (2,6-piperidinediones) possess various biological activities,^[1,2] therefore, many glutarimide derivatives have been synthesized, e.g., cycloheximide,^[3] actiketal (RK-441S),^[3] thalidomide,^[4-6] and aminoglutethimide.^[4-6,7] In particular, interest in the immuno-suppressor thalidomide has remained constant despite its teratogenicity.^[8] Thalidomide has been used in the treatment of leprosy, auto-immune diseases, and body wasting or cachexia and other disorders due to its immunomodulatory and anti-inflammatory effects. Newly found therapeutic uses include its inhibition of angiogenesis and for AIDS-related disorders.^[9,10] Thalidomide is currently used therapeutically in the racemate form. It is the classical example of a racemic drug in which one isomer, the *S*-isomer, exerts a devastating negative side effect, teratogenicity. The pure enantiomer can be obtained by preparative chiral chromatography, however, it is quickly racemizes in solution with a half life of 556 min at pH = 7.4 (37°C).^[11] Therefore, thalidomide is chirally unstable in vitro and in vivo, making the chiral purity issue moot. To take advantage of previous preclinical and clinical experiences with thalidomide, we are interested in developing a facile synthesis for thalidomide and its possible derivatives. Thalidomide has traditionally been prepared in a fairly simple three-step sequence;^[12] the last step involves a high-temperature melt reaction that yields a crude thalidomide that requires multiple recrystallizations. Here, we report the synthesis of racemic thalidomide (**1**) using a formal [3+3] cycloaddition strategy that can avoid the melt reaction, which is not amenable to standard reaction equipment.

RESULTS AND DISCUSSION

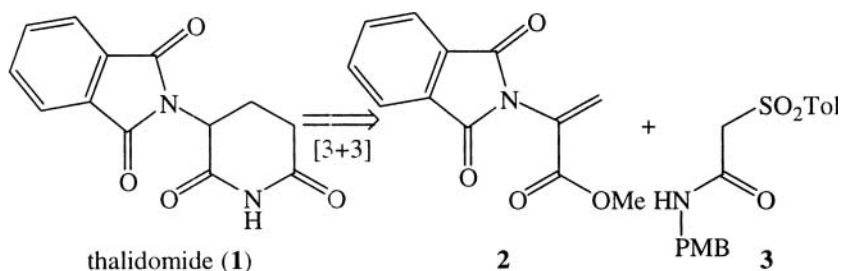
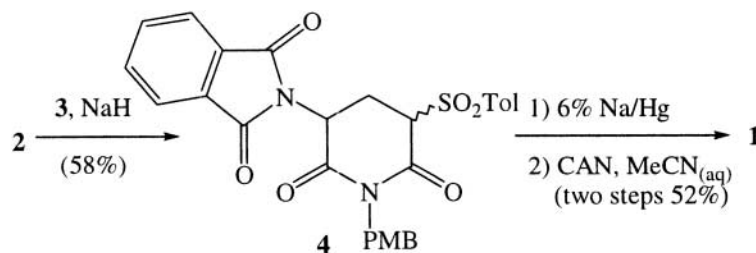
The retrosynthetic strategy for the synthesis of racemic thalidomide (**1**) is illustrated in Sch. 1. First, the glutarimide ring is established rapidly in a one-step cycloaddition reaction. The cycloaddition reaction of α -toluenesulfonyl acetamide with α - or β -substituted unsaturated esters produced various glutarimides in good yields.^[7] Second, the desulfonation and deprotection reactions of α -toluenesulfonyl glutarimides easily lead to the facile synthesis of target **1**.

Formal [3+3] cycloaddition reaction of α,β -unsaturated methyl ester **2**^[13-15] with the dianion intermediate obtained from *p*-methoxybenzyl- α -toluenesulfonyl acetamide **3** and 2.2 equiv. sodium hydride produced



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*Scheme 1.* Retrosynthesis of racemic thalidomide (1).*Scheme 2.* Synthesis of thalidomide (1) in three steps.

the 5-phthalimido α -sulfonyl glutarimide **4** in 58% yield (Sch. 2). The α,β -unsaturated methyl ester **2** was prepared from propynoic acid methyl ester and phthalimide in 78% yield.^[16] Treatment of **4** with 6% sodium amalgam (Na-Hg)^[17] and sodium phosphate in methanol produced crude PMB-thalidomide. The pH value in the buffer solution for the desulfonation of **4** must be controlled at 7.4 to prevent the ring-opening of glutarimide. Desulfonation under more basic condition (pH > 10) in absence of sodium phosphate, produced many side products. Without further purification, the crude PMB-thalidomide was treated with ceric ammonium nitrate (MeCN/H₂O = 3:1)^[18] in aliquots added over 5 h to produce racemic thalidomide **1**. Because the deprotection of PMB-thalidomide by ceric ammonium nitrate did proceed according to stoichiometric ratios, excess ceric ammonium nitrate had to be added to the reaction mixture. After six equal aliquots added at a rate of one aliquot about every hour, the PMB-thalidomide was completely transformed to **1**. The reaction progress was monitored by TLC. Thalidomide (**1**) was produced in three steps from ester **2** in 30% yield. This procedure easily produces racemic thalidomide in moderate yield on a small scale



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(ca. 5.0 mmol). The scale-up of the cycloaddition reaction to 10 g or greater create many other complex products aside from glutarimide in disproportionate ratios, thus making it difficult to efficiently synthesize thalidomide in large quantities.

CONCLUSION

The presented synthesis provides a new and simple strategy to synthesize racemic thalidomide using the formal [3+3] cycloaddition reaction. This cycloaddition method can be extended using different substituents at the 3-position of glutarimide skeleton in a one-pot reaction for synthesizing thalidomide derivatives in the search for improved activity and potency. Our method may allow for rapid synthesis of thalidomide and derivatives, but is not effective for high yield, large-scale production.^[19]

EXPERIMENTAL

General

Tetrahydrofuran was distilled prior to use from a deep-blue solution of sodium-benzophenone ketyl. All other reagents and solvents were obtained from commercial sources and used without further purification. Reactions were routinely carried out under an atmosphere of dry nitrogen with magnetic stirring. The combined organic extracts of products were dried with anhydrous magnesium sulfate before concentration in vacuo. Crude products were purified by open column chromatography using silica gel (MN Kieselgel 60, 70–230 mesh) at room temperature and normal pressure. All reported temperatures are uncorrected.

2-Phthalimido-acrylic Acid Methyl Ester (**2**)

A stirred solution of phthalimide (740 mg, 5.0 mmol), triphenylphosphine (130 mg, 0.5 mmol), and sodium acetate (210 mg, 2.5 mmol) in toluene (5 mL) was heated at refluxed temperature for 10 min. Acetic acid (150 mg, 5.0 mmol) and methyl propiolate (420 mg, 5.0 mmol) were added to above solution then were continued to reflux for 18 h. The cooled reaction mixture was chromatographed on silica gel (100 g) (hexane/ethyl acetate = 1/1, 500 mL) to yield 900 mg (78% yield) of **2**. M.p. 108–110°C;

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Electrospray-MS: $C_{12}H_9NO_4$ m/z (%) = 232 ($M^+ + 1$, 100); 1H NMR (400 MHz, $CDCl_3$) δ 7.91–7.89 (m, 2H), 7.77–7.75 (m, 2H), 6.67 (s, 1H), 5.98 (s, 1H), 3.80 (s, 3H); Anal. calcd. for $C_{12}H_9NO_4$: C, 62.34; H, 3.92. Found: C, 62.44; H, 3.96.

***N*-*p*-Methoxybenzyl-2-Toluenesulfonyl Acetamide (3)**

Chloroacetic acid chloride (1.2 g, 10.6 mmol) dissolved in tetrahydrofuran (20 mL) was added to a stirred solution of *p*-methoxybenzylamine (1.37 g, 10.0 mmol) and triethylamine (1.06 g, 10.5 mmol) in tetrahydrofuran (30 mL) at ice bath. The solution was cooled (ice bath) and stirred for 30 min then stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure to form a viscous gum at room temperature. The viscous gum was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were washed with brine (2 × 20 mL), dried over anhydrous magnesium sulfate, filtered and evaporated to give the crude α -chloroacetamide at room temperature. Without further purification, the crude product was refluxed with *p*-toluenesulfonic acid sodium salt (3.2 g, 16.5 mmol) in dioxane (70 mL) and water (70 mL) for 10 h. The mixture was concentrated under reduced pressure to obtain a brown solid at 50°C and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over anhydrous magnesium sulfate, filtered and evaporated to give the crude α -sulfonyl acetamide at room temperature. Purification on silica gel (200 g) (hexane/ethyl acetate = 1/2, 900 mL) afforded 3.0 g (90%) of **3**. Electrospray-MS: $C_{17}H_{19}NO_4S$ m/z (%) = 334 ($M^+ + 1$, 100); 1H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.3 Hz, 2H), 7.21–7.18 (m, 2H), 6.93 (brs, 1H), 6.88–6.85 (m, 2H), 4.36 (d, J = 5.8 Hz, 2H), 3.98 (s, 2H), 3.81 (s, 3H), 2.43 (s, 3H); Anal. calcd. for $C_{17}H_{19}NO_4S$: C, 61.24; H, 5.74. Found: C, 61.18; H, 5.76.

1-*p*-Methoxybenzyl-3-Phthalimido-5-Toluenesulfonyl-Piperidine-2,6-Dione (4)

A solution of acetamide **3** (333 mg, 1.0 mmol) in tetrahydrofuran (10 mL) was added to a rapidly stirred suspension of sodium hydride (60%, 88 mg, 2.2 mmol) in tetrahydrofuran (10 mL). After the mixture was stirred at room temperature for 5 min, a solution of ester **2** (231 mg, 1.0 mmol) in tetrahydrofuran (10 mL) was added. The resulting mixture was refluxed for 20 min, quenched with saturated ammonium chloride



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solution (1 mL) then concentrated under reduced pressure to give a viscous oil at room temperature. The viscous oil was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (2 × 20 mL), dried over anhydrous magnesium sulfate, filtered and evaporated to give the crude product at room temperature. Purification on silica gel (200 g) (hexane/ethyl acetate = 4/1, 600 mL) afforded 310 mg (58%) of **4** as a solid: M.p. 110–111°C; Electrospray-MS: C₂₈H₂₄N₂O₇S *m/z* (%) = 403 (100), 533 (M⁺ + 1, 42); ¹H NMR (400 MHz, CDCl₃) δ 7.89–7.74 (m, 4H), 7.53 (d, *J* = 8.2 Hz, 4/3H), 7.33–7.26 (m, 4H), 7.17 (d, *J* = 8.7 Hz, 2/3H), 6.84 (d, *J* = 8.2 Hz, 4/3H), 6.72 (d, *J* = 8.7 Hz, 2/3H), 5.78 (dd, *J* = 6.4, 13.1 Hz, 2/3H), 5.03–4.76 (m, 7/3H), 4.34 (dd, *J* = 8.4, 13.7 Hz, 1/3H), 4.21 (dd, *J* = 1.4, 6.0 Hz, 2/3H), 3.78 (s, 2H), 3.73 (s, 1H), 3.19–3.04 (m, 1H), 2.96–2.92 (m, 2/3H), 2.73–2.70 (m, 1/3H), 2.44 (s, 3H); Anal. calcd. for C₂₈H₂₄N₂O₇S: C, 63.15; H, 4.54. Found: C, 63.10; H, 4.49.

Thalidomide (**1**)

To a solution of compound **4** (133 mg, 0.25 mmol) and sodium phosphate (142 mg, 1.0 mmol) in HPLC-grade methanol (5 mL) was added 6% sodium amalgam (360 mg, 0.95 mmol). The mixture was vigorously stirred for 1 h at room temperature. After concentration of the solution under reduced pressure to give a viscous oil at room temperature, methylene chloride (10 mL) was added to the viscous oil, washed with water (4 × 10 mL), dried over anhydrous magnesium sulfate, and concentrated to get the crude desulfonyl compound. The crude compound was dissolved in acetonitrile (3 mL) and water (1 mL). Excess ceric ammonium nitrate reagent (6 eq, 1.5 mmol) was added in six equal aliquots into the solution over 5 h. The reaction was monitored after each addition using TLC. After concentrating the solution under reduced pressure, methylene chloride (3 mL) was added the residue and the mixture placed in the flash column. Purification on silica gel (50 g) (hexane/ethyl acetate = 1/2, 300 mL) afforded 34 mg (52%) of thalidomide (**1**) as a solid: M.p. 275–277°C; Electrospray-MS: C₁₃H₁₀N₂O₄ *m/z* (%) = 259 (M⁺ + 1, 100); ¹H NMR (400 MHz, CDCl₃) δ 7.99 (brs, 1H), 7.89–7.86 (m, 2H), 7.78–7.75 (m, 2H), 4.98 (dd, *J* = 5.3, 12.4 Hz, 1H), 2.93–2.71 (m, 3H), 2.18–2.12 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 170.74, 167.89, 167.33, 134.59 (2x), 134.44, 131.82, 123.91 (2x), 123.80, 49.43, 31.49, 22.3. Anal. calcd. for C₁₃H₁₀N₂O₄: C, 60.47; H, 3.90. Found: C, 60.41; H, 3.86.



ACKNOWLEDGMENT

The authors would like to thank the National Science Council of the Republic of China for financial support.

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Received in the USA June 15, 2002

Thiothalidomides: Novel Isosteric Analogues of Thalidomide with Enhanced TNF- α Inhibitory Activity[†]

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Received April 4, 2003

Thalidomide is being increasingly used in the clinical management of a wide spectrum of immunologically-mediated and infectious diseases, and cancers. However, the mechanisms underlying its pharmacological action are still under investigation. In this regard, oral thalidomide is clinically valuable in the treatment of erythema nodosum leprosum (ENL) and multiple myeloma and effectively reduces tumor necrosis factor- α (TNF- α) levels and angiogenesis *in vivo*. This contrasts with its relatively weak effects on TNF- α and angiogenesis in *in vitro* studies and implies that active metabolites contribute to its *in vivo* pharmacologic action and that specific analogues would be endowed with potent activity. Our focus in the structural modification of thalidomide is toward the discovery of novel isosteric active analogues. In this regard, a series of thiothalidomides and analogues were synthesized and evaluated for their TNF- α inhibitory activity against lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC). This was combined with a PBMC viability assay to differentiate reductions in TNF- α secretion from cellular toxicity. Two isosteric analogues of thalidomide, compounds **15** and **16**, that mostly reflect the parent compound, together with the simple structure, dithioglutarimide **19**, potently inhibited TNF- α secretion, compared to thalidomide, **1**. The mechanism underpinning this most likely is posttranscriptional, as each of these compounds decreased TNF- α mRNA stability via its 3'-UTR. The potency of **19** warrants further study and suggests that replacement of the amide carbonyl with a thiocarbonyl may be beneficial for increased TNF- α inhibitory action. In addition, an intact phthalimido moiety appeared to be requisite for TNF- α inhibitory activity.

Introduction

Thalidomide (*N*- α -phthalimidoglutarimide (**1**) (Figure 1)) is a glutamic acid derivative that was introduced onto the market as a sedative hypnotic in 1956, but was withdrawn in 1961 due to the development of severe congenital abnormalities in babies born to mothers using it for morning sickness.¹ Interest in the agent was reawakened after thalidomide was found clinically effective in the treatment of erythema nodosum leprosum (ENL)² and in the treatment of HIV wasting syndrome and various cancers.^{3,4} Mechanistic studies of its ENL activity demonstrated an anti-TNF- α action.⁵ Specifically, thalidomide enhances the degradation of tumor necrosis factor- α (TNF- α) RNA and, thereby,

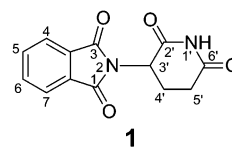


Figure 1. The structure of thalidomide.

lowers its synthesis and secretion.^{6,7} Further studies have defined it to be a costimulator of both CD8+ and CD4+ T cells,⁸ an inhibitor of angiogenesis via its inhibitory actions on basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF),^{9,10} and an inhibitor of the transcription factor, NF κ B.^{6,7} However, it is the action of thalidomide on TNF- α that provides the focus of the current study.

TNF- α and family members play pivotal roles in a variety of physiological and pathological processes, which include cell proliferation and differentiation, apoptosis, the modulation of immune responses, and induction of inflammation. TNF- α acts via two receptors, TNFR1 and 2. The former is expressed in all tissues and is the predominant signaling receptor for TNF- α . The latter is primarily expressed on immune cells and mediates more limited biological responses. The exposure of cells to TNF- α can result in activation

[†] Dedicated to our wonderful collaborator, mentor, and friend, Arnold Brossi, on his 80th birthday.

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of a caspase cascade leading to cell death via apoptosis. Indeed, major cell surface molecules capable of initiating apoptosis are members of the TNF family of ligands and receptors. For example, death-inducing members of the TNF receptor family each contain a cytoplasmic 'death domain' (DD), which is a protein-protein interaction motif critical for engaging downstream components of the signal transduction machinery.¹¹ Recently, TRAIL, the tumor necrosis factor-related apoptosis-inducing ligand, has been shown to selectively induce apoptosis of tumor cells, but not most normal cells.¹² It is suggested that TRAIL mediates thymocyte apoptosis and is important in the induction of autoimmune diseases. More often, however, TNF- α receptor binding induces the activation of transcription factors, AP-1 and NF κ B, that thereafter induce genes involved in acute and chronic inflammatory responses.^{13,14} Overproduction of TNF- α has thus been implicated in many inflammatory diseases, such as rheumatoid arthritis, graft-versus-host disease, and Crohn's disease, and it additionally exacerbates ENL, septic shock, AIDS, and dementia associated with Alzheimer's disease (AD). In this context, our present work, which examines novel analogues of thalidomide with enhanced TNF- α inhibitory activity, assumes significance.

The mechanisms underlying thalidomide's diverse actions, together with identification of the active species, remains an area of intense research. The compound is more active in vivo than would be predicted from its potency in in vitro studies.^{15,16} This suggests that active metabolites largely account for its in vivo activity¹⁷ and that specific analogues of thalidomide would be endowed with high potency. Indeed, seldom has such a simple molecule had such a controversial history and held so much therapeutic promise.^{3,4,10,18} The focus of our research has been two pronged: (i) to elucidate the action of thalidomide on the regulation of TNF- α levels, and (ii) to develop analogues of clinical potential that potently inhibit TNF- α secretion.

A number of papers have been published on the design and synthesis of thalidomide analogues optimized to reduce TNF- α synthesis.¹⁹⁻²⁵ These have primarily focused on exploring structural modifications of the phthaloyl ring or glutarimide ring of thalidomide and have described *N*-phthaloyl 3-amino-3-arylpropionic acid derivatives, amino-phthaloyl-substituted and tetrafluorophthaloyl-substituted analogues of thalidomide, and *N*-substituted phthalimides with a simplified glutarimide moiety. In addition, following the demonstration that the antiangiogenic property of thalidomide is associated with its hydroxylated metabolites, the open ring metabolites,^{15,26} syntheses of the hydroxylated and hydrolysis metabolites as inhibitors of angiogenesis or tumor metastasis have been reported.^{23,24,27} In the current paper we focus on the synthesis and evaluation of novel sulfur analogues of thalidomide with enhanced biological activity.

Although extensive studies exist regarding the structure-activity relationships between thalidomide and TNF- α , very little is known about the contribution of four amide carbonyl groups of thalidomide to its biological activity. The isosteric replacement of carbonyl group by a thiocarbonyl group provides isosteric analogues of thalidomide: thiothalidomides. A series of thiothalido-

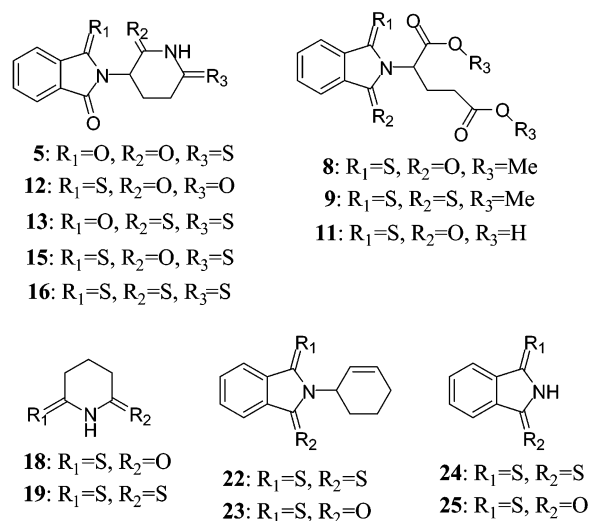


Figure 2. The structures of thiothalidomides and analogues.

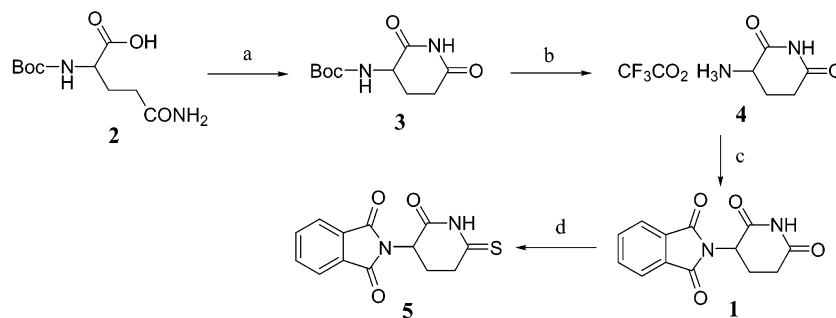
mides and analogues were designed to explore their action on inhibition of TNF- α (Figure 2).

Chemistry

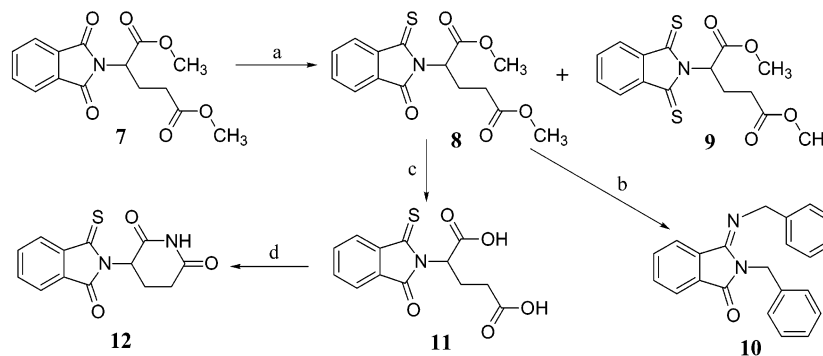
Monothiothalidomide **5** was prepared as shown in Scheme 1. *tert*-Butoxycarbonyl-L-glutamine **2** was refluxed with carbonyldiimidazole (CDI) in THF and cyclized to afford imide **3**.²¹ Imide **3** then was treated with trifluoroacetic acid in CH₂Cl₂ to remove the protective group to generate aminoglutaramide trifluoroacetate **4**. Without further purification, compound **4** was reacted with phthalic anhydride in refluxing THF in the presence of triethylamine to produce thalidomide **1** in the total yield of 31% from compound **2**. Thalidomide **1** was thionated with Lawesson's reagent (LR)²⁸ to generate a single new product, whose structure was identified as 6'-thiothalidomide **5** by MS and 1D and 2D NMR spectra. The position of thiocarbonyl group was established from the HMBC cross-peak of H-5'/C-6' (see Supporting Information: HMBC spectrum of compound **5**).

The synthesis of 3-thiothalidomide **12** is shown in Scheme 2. *N*-Phthaloyl-L-glutamic acid **6** was esterified to afford diester **7**. Compound **7** was thionated with LR at 110 °C to give compound **8** as a major product. Concurrently, compound **9** was separated as a minor product by chromatography. However, no thioesters were separated from the reaction mixture, as any ester reacts with LR at 130 °C.²⁷ Furthermore, we failed to obtain 3-thiothalidomide, **12**, through the cyclization of compound **8** with ammonia or amine, as ammonia reacts with the thioamide; reaction of compound **8** with benzylamine produced the unexpected compound **10**. In an alternative approach, compound **8** was hydrolyzed under acidic conditions to give diacid **11**. Compound **11** was then reacted with trifluoroacetamide to generate 1-thiothalidomide **12** in the presence of 1-hydroxybenzotriazole (HOBT) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI).²⁹

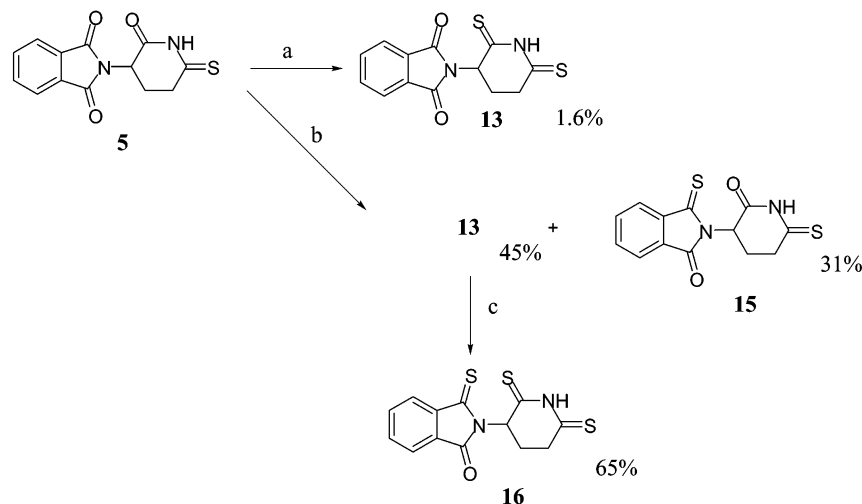
In the synthesis of dithiothalidomide, our initial attempt involved the reaction of monothiothalidomide with LR at reflux in toluene. Under such conditions, 2',6'-dithiothalidomide was obtained in a yield of less than 2% (Scheme 3(a)). Such a yield was so low that

Scheme 1^a

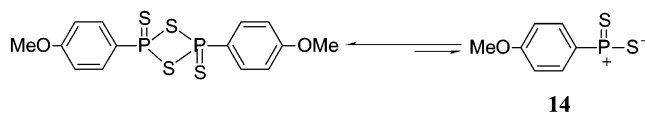
^a Reagents: (a) CDI/THF; (b) CF₃COOH/CH₂Cl₂; (c) phthalic anhydride, Et₃N/THF; (d) Lawesson's reagent/toluene.

Scheme 2^a

^a Reagents: (a) Lawesson's reagent/toluene; (b) benzylamine; (c) HCl/HOAc; (d) F₃C₃CONH₂, HOBT, EDCI, Et₃N/CH₂Cl₂.

Scheme 3^a

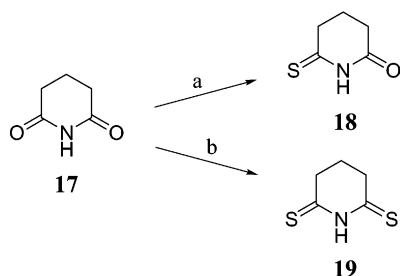
^a Reagents: (a) Lawesson's reagent/toluene; (b) Lawesson's reagent, pyridine/toluene; (c) Lawesson's reagent, morpholine/toluene.

Scheme 4. Mechanism of Catalysis for Lawesson's Reagent

improvement was clearly essential and was undertaken by modifying the reaction conditions. It is believed that the mechanism underlying the reaction between LR and a carbonyl moiety is that a highly reactive dithiophosphine ylide **14**, rather than LR itself, likely is the active thionating agent (Scheme 4).²⁸ The Lewis base should be able to increase the reactivity of LR as the base may drive the unfavorable equilibrium and elevate the

concentration of the ylide **14**. When pyridine was used as a catalyst for thionation, monothiothalidomide **5** was thionated with LR to produce two dithiothalidomides, **13** and **15**, in the yields of 45% and 31%, respectively (Scheme 3b,c). Dithiothalidomide **13** could be further thionated with LR in the presence of the stronger base, morpholine, to give trithiothalidomide **16** in the yield of 65%.

Glutarimide **17** was thionated with LR in THF at room temperature to afford compound **18** as a major product.³⁰ Glutarimide **17** also was refluxed with LR in toluene to produce dithioglutarimide **19** (Scheme 5).³¹ Reaction of potassium phthalimide with 3-bromocyclohexene underwent the Gabriel reaction to give compound **21**. Thereafter, thionation of compound **21** with

Scheme 5^a

^a Reagents: (a) Lawesson's reagent/THF, rt; (b) Lawesson's reagent, reflux/toluene.

LR afforded compounds **22** and **23** (Scheme 6). Compounds **24** and **25** were prepared in a similar procedure to the preparation of compounds **22** and **23**.³²

Results and Discussion

The action of the described thiothalidomide analogues to inhibit TNF- α secretion was assessed in human peripheral blood mononuclear cells (PBMC) and is shown in Table 1. Thalidomide, **1**, entirely lacked activity at 30 μ M. A concentration of 100 μ M was required for significant activity. The monothiothalidomides, 6'-thiothalidomide **5**, and 3-thiothalidomide **12** showed only marginal activity at 30 μ M with 31% and 23% inhibition of TNF- α secretion, respectively. In contrast, the dithiothalidomides, including 2',6'-dithiothalidomide **13** and 3,6'-dithiothalidomide **15**, exhibited more potent inhibitory activities with IC₅₀ values of 20 μ M and 11 μ M, respectively. However, assessment of cell viability by MTS assay showed that **13** induced increasing cytotoxicity at higher concentrations. Trithiothalidomide **16** inhibited TNF- α production with an IC₅₀ of 6 μ M without accompanying toxicity. Compared with thalidomide, **1**, with an IC₅₀ of \sim 200 μ M for the inhibition of TNF- α synthesis, trithiothalidomide **16** is over 30-fold more active. Hence, successive replacement of a carbonyl with a thiocarbonyl group led to improved inhibitory activity compared to **1**, unassociated with toxicity. In this regard, the synthesized thiothalidomides possessed TNF- α lowering potency in the following decreasing order: trithiothalidomide **16** > dithiothalidomide **15** and **13** > monothiothalidomides **5** and **12** > thalidomide, **1**.

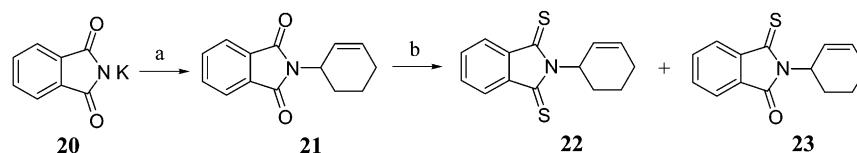
A comparison of the physical properties of thalidomide, **1**, and thiothalidomides shows that they have similar van der Waals radii and bond angles, although the C=S bond is slightly longer than the C=O bond. A possible explanation accounting for the elevated potency of the thiothalidomides is that their enhanced lipophilicity and loss of hydrogen bond acceptor capability potentially allows the attainment of higher intracellular drug levels. Interestingly, compounds **8**, **9**, and **11** are thio analogues of hydrolysis metabolites of thalidomide. Assessment of their TNF- α inhibitory action determined that the monothio analogue, **8**, has an IC₅₀ of 20 μ M without toxicity; demethylation (**11**) lowered potency. The dithio analogue, **9**, proved 2-fold more potent still than **8**, but induced cellular toxicity at lower concentrations. Intriguingly, thio analogues **22** and **23**, with a simplified glutarimide ring, were found to be active TNF- α inhibitors, albeit with some toxicity at 30 μ M, with IC₅₀ values (15 μ M and 16 μ M, respectively) that

were greater than **12** (>30 μ M) possessing a normal glutarimide ring.

In this regard, thalidomide is composed of two distinct moieties: the glutarimide and phthalimide rings. Thio-glutarimides and thiophthalimides were thus synthesized and evaluated to assess the effect of thio-analogues of these two moieties on TNF- α levels. Monothio-glutarimide **18** minimally inhibited TNF- α secretion at a concentration of 30 μ M; however, dithio-glutarimide **19** exerted a potent inhibitory effect with an IC₅₀ of 8 μ M and a lack of toxicity. An intact phthaloyl ring is believed to be required for the inhibition of TNF- α secretion.¹⁹ Surprisingly, such a simple structure, dithio-glutarimide **19**, proved to be 25-fold more active than **1**. In contrast, 2',6'-dithiothalidomide **13**, a phthalimido-substituted dithio-glutarimide, is less active than dithio-glutarimide **19** and induces toxicity at high concentration. Monothio-phthalimide **25** showed a marginal TNF- α activity at a concentration of 30 μ M without toxicity. Interestingly, however, dithio-phthalimide **24** was found to possess potent activity with an IC₅₀ of 3 μ M. Although it was associated with toxicity at 30 μ M, its inhibition of TNF- α occurred at a log lower concentration that was well tolerated.

As described, compounds **15**, **16**, and **19** potently inhibited TNF- α secretion without toxicity. As a consequence, additional studies were undertaken to elucidate the mechanism underpinning this action. Gene and protein expressions are controlled at the level of transcription, posttranscription, RNA stability, and translation under different physiological stimuli. Recently, posttranscriptional pathways have been recognized to provide a major means of regulating eukaryotic gene expression. In this regard, TNF- α and other cytokines and protooncogenes are known to be regulated at the posttranscriptional level.^{6,7,33,34} Multiple proteins, including the four cloned proteins AUF1, HuR, TTP, and HuD have been shown to bind to a region of the mRNA that contains adenylate/uridylate (AU)-rich elements (AREs) in the 3'-untranslated region (UTR). These proteins mediate RNA turnover and decay and hence translational efficiency.^{35,36} The stability of TNF- α mRNA is largely regulated at its 3'-UTR, which contains a well characterized ARE. Although AREs are found in a number of different cytokine and protooncogene RNAs, the pathways by which they induce degradation are highly specific for a given ARE, suggesting some cellular specificity. When the AREs from different cytokines are complexed with AUF1, different binding affinities are observed. Notably, however, the highest affinity for AUF1 is to human and then mouse TNF- α .

To determine the involvement of the 3'-UTR in the action of our thalidomide analogues, their ability was assessed to inhibit reporter gene activity in cells containing the TNF- α 3'-UTR versus a control vector (Figure 3). Specifically, this cell-based assay comprised of two stably transfected cell lines derived from the mouse macrophage line, RAW264.7. One line, designated "luciferase only", expressed a luciferase reporter construct without any UTR sequences, whereas the other, designated "luciferase + TNF UTRs", expressed a luciferase reporter construct with the entire 3'-UTR of human TNF- α inserted directly downstream of the luciferase coding region. Compounds **15**, **16**, and **19**

Scheme 6^a

^a Reagents: (a) 3-bromocyclohexene/DMF; (b) Lawesson's reagent/toluene.

Table 1. Inhibition of LPS-Induced TNF- α Production in PBMC and Cell Viability^a

compd	% inhibition		cell viability		
	at 30 μ M	IC ₅₀ (μ M)	at 30 μ M	at 3 μ M	at 0.3 μ M
5	31	>30	>100	90	96
8	56	20	93	99	96
9	85	10	57	86	89
11	20	>30	86	93	93
12	23	>30	94	100	94
13	52	20	69	87	94
15	61	11	>100	87	94
16	79	6	94	86	90
18	15	>30	>100	84	86
19	75	8	>100	98	99
22	86	15	50	94	96
23	85	16	57	89	99
24	95	3	54	83	83
25	34	>30	>100	94	94

^a Thalidomide (**1**) totally lacked activity at 30 μ M (IC₅₀ ~ 200 μ M).

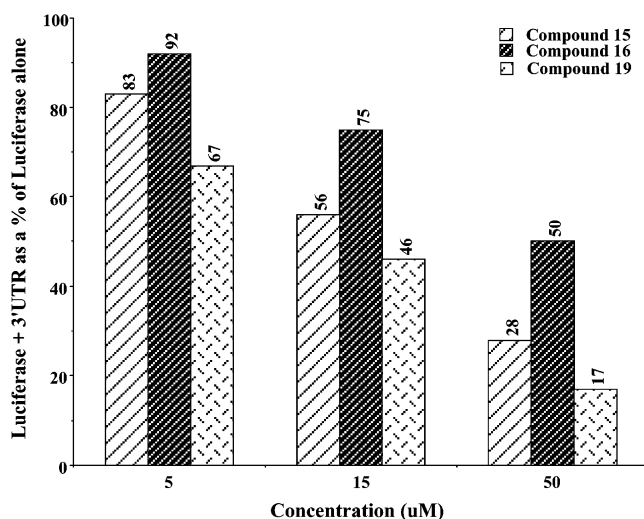


Figure 3. The action of compounds **15**, **16**, and **19** in cells (mouse macrophage cell line, RAW264.7.) possessing a luciferase reporter element plus the 3'-UTR of human TNF- α compared to cells lacking the 3'-UTR. All agents lowered luciferase reporter activity in cells stably expressing the 3'-UTR. Thalidomide **1** lacked activity at 50 μ M.

exerted differential effect on the two cell lines in a dose-dependent manner, consistent with their ability to inhibit TNF- α production via the 3'-UTR.

As TNF- α protein levels changed without significant alterations in mRNA levels (data not shown), protein expression is presumably regulated via translational control (at the posttranscriptional level). There is precedence for translational (protein) control through either the 3'- or 5'-UTR regions of a number of critical proteins that are current drug targets. For example, levels of the β -amyloid precursor protein (APP) that is central to the development of AD can be regulated by either UTR. Turnover and translation of APP mRNA is regulated by a 29-nucleotide instability element within the 3'-

UTR, located 200 nucleotides downstream from the stop codon.³⁷ This 3'-UTR element acts as an mRNA destabilizer whose function can be inhibited by the presence of growth factors. In contrast, different cytokines,³⁸ including TNF- α ³⁹ and iron,⁴⁰ can up regulate APP protein synthesis at the level of its 5'-UTR, whereas, interestingly, the anticholinesterase, phenserine, that is currently in clinical trials for AD lowers APP protein levels with concurrent maintenance of mRNA steady-state levels through translational modification within the same 5'-UTR element.⁴¹ A further example is that of the human immunodeficiency virus 1 (HIV-1) Trans-activating transduction (tat) protein, which binds trans-activation-responsive region (TAR) RNA.⁴² Tat is brought into contact with the transcription machinery after binding the TAR element, which is a 59-residue stem-loop RNA found at the 5' end of all HIV-1 transcripts.⁴² Finally, thalidomide (**1**) has been reported to lower cyclooxygenase-2 (Cox-2) biosynthesis via its 3'-UTR⁴³ that appears to likewise contain an ARE that can regulate Cox-2 mRNA stability.⁴⁴ Our studies of analogues **15**, **16**, and **19** confirm the regulation of TNF- α protein levels by thalidomide (**1**) via its 3'-UTR, whether the 5'-UTR contains a similar element that is accessible to pharmacological manipulation remains to be determined, as does action against Cox-2.

Our future studies are aimed at assessing whether the in vitro activity of the described thalidomide analogues translates into pertinent in vivo models, such as in mice challenged with LPS.³³ In terms of the mechanism of TNF- α signaling, there is recent in vivo evidence showing that the AU-rich 3'-UTRs of TNF- α and IL-6 are downstream to MAPKAP kinase 2 (MK2) signaling. MK2 is an essential component of mechanisms that regulate biosynthesis of IL-6 at the level of mRNA stability, and of TNF- α mainly through TNF-ARE-dependent translational control.⁴⁵ In addition, future in vitro studies involving the induction of other cytokines and deletion of the ARE will define the specificity of the most potent compounds described herein, as well as ARE-independent actions. Knockout mice for the TNF ARE or the destabilizing protein TTP both have elevated levels of TNF- α and increased inflammation. Hence, the interaction between the TNF- α ARE and the cognate RNA binding proteins appears to represent an attractive target for regulation at the posttranscriptional level.

In conclusion, we have synthesized a novel series of thiothalidomide analogues that are more potent inhibitors of TNF- α production in LPS-induced human PBMCs than thalidomide, **1**. The isosteric replacement of successive carbonyl groups by a thiocarbonyl leads to an increasing inhibition with the number moieties replaced (trithiothalidomide **16** > dithiothalidomide **15** and **13** > monothiothalidomides **5** and **12** > thalidomide **1**). Preliminary studies indicate that the induced TNF- α inhibition involved posttranscriptional mechanisms and

occurred via its 3'-UTR. Like thalidomide **1**, **15**, and **16** effectively inhibited angiogenesis, whereas the potent TNF- α inhibitor, dithioglutarimide **19**, was inactive in angiogenesis assays (data not shown); suggesting that different structure/activity relations are responsible for these pharmacological actions. TNF- α has been validated as a drug target with Remicade (Cetecor, Malvern, PA; Schering-Plough, Orange, NJ) and Enbrel (Amgen, Thousand Oaks, CA; Wyeth-Ayerst, Princeton, NJ) on the market; however, both are large macromolecules and hence require injection. The development of small, druglike molecules to potently and safely inhibit TNF- α likely will be of significant clinical value.

Experimental Section

General. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. ^1H NMR, ^{13}C NMR, and 2D NMR were recorded on a Bruker AC-300 spectrometer. Mass spectra and high-resolution mass spectra (HRMS) were recorded on a VG 7070 mass spectrometer and a Agilent Technologies 5973N GC-MS (CI). All exact mass measurements show an error of less than 5 ppm. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

3-(tert-Butoxycarbonylamino)-2,6-piperidinedione (3). A mixture of *N*-(tert-butoxycarbonyl)-L-glutamine (4.92 g, 20 mmol) and carbonyl diimidazole (3.24 g, 20 mmol) in THF (100 mL) was refluxed for 16 h. Thereafter, solvent was removed, and the crude product was recrystallized from hot EtOAc to give compound **3** (2.04 g, 45%) as white crystals: mp 214–215 °C; ^1H NMR (DMSO- d_6) δ 4.22 (dd, $J = 6.2$ Hz, $J = 11.0$ Hz, 1H), 2.77–2.65 (m, 1H), 2.45 (m, 1H), 1.96–1.87 (m, 2H), 1.40 (s, 9H); MS (CI/CH₄) m/z 227 [M - 1]⁺.

2-(2-Oxo-6-thioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione (5). Compound **3** (1.14 g, 5 mmol) was suspended in CH₂Cl₂ (100 mL). To the mixture was added CF₃COOH (10 mL), and this then was stirred at room temperature for 4 h. The solvent was evaporated to give crude **4** (1.25 g): ^1H NMR (DMSO- d_6) δ 11.42 (s, 1H), 8.70 (br, 2H), 4.31 (dd, $J = 5.4$ Hz, $J = 13$ Hz), 2.88–2.72 (m, 2H), 2.25–2.09 (m, 2H). A mixture of crude **4** (1.25 g), phthalic anhydride (0.89 g, 6 mmol), and Et₃N (1.39 mL, 10 mmol) in THF (150 mL) was refluxed for 2 days. The reaction mixture was concentrated, and the residue was crystallized from ethyl acetate to give thalidomide (**1**) (0.89 g, 69%) as white crystals; mp 276 °C (lit. 276–279 °C). The mixture of thalidomide **1** (258 mg, 1 mmol) and Lawesson's reagent (222 mg, 0.55 mmol) in toluene (50 mL) was stirred at reflux for 12 h; thereafter, solvent was removed under vacuum. The resulting residue was purified by column chromatography using CH₂Cl₂ as the eluent to afford compound **5** (200 mg, 73%) as a yellow solid: mp 225–226 °C; ^1H NMR (DMSO- d_6) δ 12.83 (s, 1H, NH), 8.00–7.92 (m, 4H, Ph), 5.32 (dd, $J = 5.6$ Hz, $J = 12.9$ Hz, 1H, H-3'), 3.28–3.25 (m, 2H, H-5'), 2.60–2.54 (m, 1H, H-4'), 2.17–2.10 (m, 1H, H-4'); ^{13}C NMR (DMSO- d_6) δ 208.7(C-6'), 165.3(C-2'), 165.2(C-1 and C-3), 133.1(C-5 and C-6), 129.3 (C-3a, C-7a), 121.7 (C-4 and C-7), 46.9 (C-3'), 38.9 (C-5'), 21.79 (C-4'); MS (CI/CH₄) m/z 274 (M⁺); Anal. (C₁₃H₁₀N₂O₃S) C, H, N.

Dimethyl 2-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)pentanedioate (7). To a solution of *N*-phthaloyl-L-glutamic acid (200 mg, 0.72 mmol) in methanol (10 mL) was added, dropwise, thionyl chloride (1 mL). The reaction mixture was refluxed for 6 h. The solvent was removed under reduced pressure, dissolved in ethyl acetate (100 mL), and then washed with saturated aqueous Na₂CO₃ solution (2 \times 30 mL) and water (2 \times 30 mL). The ethyl acetate layer was dried over Na₂SO₄ and then evaporated, leaving an oil, which upon purification by silica gel chromatography, using CH₂Cl₂:EtOAc (1:1) as the eluent, gave compound **7** (161 mg, 73%) as an oil; ^1H NMR (CDCl₃) δ 7.87–7.84 (m, 2H), 7.75–7.72 (m, 2H), 4.91 (dd, $J = 5$ Hz, $J = 9$ Hz, 1H), 3.73 (s, 3H), 3.62 (s, 3H), 2.67–2.56 (m, 1H), 2.51–2.44 (m, 1H), 2.41–2.35 (m, 2H).

Dimethyl 2-(1,3-dihydro-1-oxo-3-thioxo-2H-isoindol-2-yl)pentanedioate (8) and Dimethyl 2-(1,3-dihydro-1,3-

dithio-2H-isoindol-2-yl)pentanedioate (9). A mixture of compound **7** (144 mg, 0.47 mmol) and LR (191 mg, 0.47 mmol) in toluene was stirred in a 110 °C oil bath for 10 h. The solvent was then evaporated, and the residue was purified by column chromatography (silica gel) using CH₂Cl₂ as the eluent, to obtain compound **9** (17 mg, 11%) as a dark red oil. Thereafter, using CH₂Cl₂:EtOAc (10:1) as the eluent the more polar component **8** (105 mg, 70%) was obtained as a red oil.

Compound **8**: ^1H NMR (CDCl₃) δ 7.98–7.96 (m, 1H), 7.81–7.70 (m, 3H), 5.53 (dd, $J = 5.1$ Hz, $J = 10$ Hz, 1H), 3.70 (s, 3H), 3.59 (s, 3H), 2.76–2.56 (m, 2H), 2.40–2.33 (m, 2H); MS (CI/CH₄) m/z 321 (M⁺).

Compound **9**: ^1H NMR (CDCl₃) δ 7.87–7.84 (m, 2H), 7.73–7.68 (m, 2H), 6.09 (dd, $J = 5$ Hz, $J = 10$ Hz, 1H), 3.70 (s, 3H), 3.58 (s, 3H), 2.81–2.63 (m, 2H), 2.40–2.24 (m, 2H); MS (DEI) m/z 337 (M⁺); HRMS (DEI) calcd for C₁₅H₁₅N₂O₄S₂ 337.0442 (M⁺), found 337.0449.

2-(1,3-Dihydro-1-oxo-3-thioxo-2H-isoindol-2-yl)pentanedioic Acid (11). Compound **8** (350 mg, 1.09 mmol) was stirred with a 1:1 mixture of acetic acid glacial and concentrated HCl in a 100 °C oil bath for 2.5 h. Ethyl acetate (100 mL) and ice-water (30 mL) was added. The ethyl acetate layer was separated, washed with ice-water, dried over Na₂SO₄, and concentrated. The resulting syrup was crystallized with ether to afford compound **11** as red crystals (253 mg, 79%); mp 157 °C; ^1H NMR (DMSO- d_6) δ 8.04–7.96 (m, 1H), 7.91–7.74 (m, 3H), 5.43 (dd, $J = 5.1$ Hz, $J = 9.6$ Hz, 1H), 2.42–2.33 (m, 2H), 2.30–2.26 (m, 2H); MS (DEI) m/z 293 (M⁺); HRMS (DEI) calcd for C₁₃H₁₁N₂O₅S 293.0358 (M⁺), found 293.0363; Anal. (C₁₃H₁₁N₂O₅S) H, N; C: calcd, 53.24; found, 53.88.

2,3-Dihydro-3-thioxo-2-(2,6-dioxo-3-piperidinyl)-1H-isoindol-1-one (12). A mixture of compound **8** (81 mg, 0.276 mmol), trifluoroacetamide (57 mg, 0.50 mmol), 1-hydroxybenzotriazole (145 mg, 1.07 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (200 mg, 1.04 mmol), and triethylamine (0.21 mL, 1.51 mmol) in CH₂Cl₂ (1.5 mL) was stirred at ambient temperature for 3 days. Water (10 mL) and CH₂Cl₂ (10 mL) were added. The dichloromethane layer was separated, washed with water, dried over Na₂SO₄, and evaporated under reduced pressure. Purification by chromatography, with EtOAc:CH₂Cl₂ (1:10) as the eluent, gave compound **12** (48 mg, 63%) as a red solid: mp 255 °C; ^1H NMR (CDCl₃) δ 8.00–7.98 (m, 1H), 7.80–7.71 (m, 3H), 5.63 (br, 1H), 2.98–2.70 (m, 3H), 2.18–2.15 (m, 1H); MS (CI/CH₄) m/z 274 (M⁺); Anal. (C₁₃H₁₀N₂O₃S) C, H, N.

2-(2,6-Dithio-3-piperidinyl)-1H-isoindole-1,3(2H)-dione (13) and 2,3-Dihydro-3-thioxo-2-(2-oxo-6-thioxo-3-piperidinyl)-1H-isoindol-1-one (15). The mixture of **5** (146 mg, 0.533 mmol), LR (108 mg, 0.267 mmol), and pyridine (21 μL) in toluene was stirred at 110 °C under an atmosphere of N₂ for 12 h. Thereafter, additional LR (108 mg, 0.267 mmol) and pyridine (21 μL) were added, and the reaction mixture was stirred for a further 12 h. The solvent was removed under vacuum, and the residue was purified by column chromatography with CH₂Cl₂:petroleum ether (2:1, 10:1) and then CH₂Cl₂:EtOAc (10:1) as eluents to afford **13** (30 mg, 45%), **15** (21 mg, 31.5%), and starting material **5** (83 mg).

Compound **13** (yellow solid): mp 263–265 °C; ^1H NMR (CDCl₃) δ 7.78–7.74 (m, 2H), 7.66–7.63 (m, 2H), 5.00 (dd, $J = 4.9$ Hz, 11.9 Hz, 1H), 3.43–3.35 (m, 1H), 2.95–2.84 (m, 2H), 2.08–2.06 (m, 1H); MS (DEI) m/z 290 (M⁺); HRMS (DEI) calcd for C₁₃H₁₀N₂O₂S₂ 290.0184 (M⁺), found 290.0185; Anal. (C₁₃H₁₀N₂O₂S₂) C, H, N.

Compound **15** (red solid): mp 240–242 °C; ^1H NMR (CDCl₃) δ 9.44 (s, 1H), 8.05–8.02 (m, 1H), 7.86–7.76 (m, 3H), 5.75–5.64 (m, 1H), 3.57–3.52 (m, 1H), 3.09–2.99 (m, 2H), 2.19–2.12 (m, 1H). ^{13}C NMR (DMSO): 208.16, 207.98, 166.10, 165.39, 134.32, 133.11, 132.42, 124.30, 122.15, 121.11, 49.64, 21.29; MS (DEI) m/z 291 (MH⁺); HRMS (DEI) calcd for C₁₃H₁₁N₂O₂S₂ 291.0262 (M⁺), found 291.0264; Anal. (C₁₃H₁₀N₂O₂S₂·0.5H₂O) C, H, N.

2,3-Dihydro-3-thioxo-2-(2,6-dithio-3-piperidinyl)-1H-isoindol-1-one (16). The mixture of compound **13** (29 mg, 0.1 mmol), LR (22 mg, 0.054 mmol), and morpholine (9 μL , 0.1

mmol) in toluene (10 mL) was stirred at reflux under an atmosphere of N₂ for 16 h. The solvent was removed under vacuum, and the residue was purified by column chromatography using CH₂Cl₂:petroleum ether (1:1) as the eluent to afford compound **16** (20 mg, 65%) as a red solid: mp 244 °C; ¹H NMR (CDCl₃) δ 10.81 (s, 1H), 8.05–8.01 (m, 1H), 7.91–7.75 (m, 3H), 5.92 (m, 1H), 3.57–3.52 (m, 1H), 3.13–2.97 (m, 2H), 2.18–2.15 (m, 1H); MS(DEI) *m/z* 306 (M⁺); HRMS (DEI) calcd for C₁₃H₁₀N₂OS₃ 305.9955 (M⁺), found 305.9951; Anal. (C₁₃H₁₀N₂OS₃·0.5H₂O) C, H, N.

6-Thioxo-2-piperidinone (18). The mixture of glutarimide (0.45 g, 4 mmol) and LR (0.809 g, 2 mmol) in THF (30 mL) was stirred at room temperature for 2 days. The solvent was evaporated under vacuum, and the residue was purified by column chromatography using petroleum ether:EtOAc (1:1) as the eluent to give compound **18** as a yellow solid (0.361 g, 70%): mp 135 °C; ¹H NMR (CDCl₃) δ 2.96 (t, *J* = 5.7 Hz, 2H), 2.58 (t, *J* = 5.8 Hz, 2H), 1.96 (m, 2H); MS (CI/CH₄) *m/z* 129 (M⁺); Anal. (C₅H₇NOS) C, H, N.

2,6-Piperidinedithione (19). The mixture of glutarimide (0.34 g, 3 mmol) and LR (1.22 g, 3 mmol) in toluene (30 mL) was stirred at reflux for 3 h. The solvent was evaporated under vacuum, and the residue was purified by column chromatography using petroleum ether:EtOAc (20:1) as the eluent to give compound **19** as a yellow solid (0.286 g, 66%): mp 103 °C; ¹H NMR (CDCl₃) δ 3.02 (t, *J* = 6.3 Hz, 4H), 1.98 (t, *J* = 6.3 Hz, 2H); MS (CI/CH₄) *m/z* 145 (M⁺); Anal. (C₅H₇NS₂) C, H, N.

2-(3-Cyclohexenyl)-1H-isoindole-1,3(2H)-dione (21). A mixture of potassium phthalimide (1.85 g, 3 mmol) and 3-bromocyclohexene (1.79 g, 3 mmol) in DMF (15 mL) was stirred in a 100 °C oil bath for 12 h. The cooled reaction mixture was poured into ice–water. The solid was collected by filtration and purified by flash chromatography with CH₂-Cl₂ as the eluent to afford compound **21** (1.6 g, 72%) as pink crystals; mp 114 °C; ¹H NMR (CDCl₃) δ 7.73–7.69 (m, 2H), 7.62–7.58 (m, 2H), 5.85–5.82 (m, 1H), 5.47–5.44 (m, 1H), 4.80–4.78 (m, 1H), 2.14–2.00 (m, 3H), 1.86–1.78 (m, 2H), 1.64–1.58 (m, 1H).

2-(3-Cyclohexenyl)-1H-isoindol-1,3(2H)-dithione (22) and 2,3-Dihydro-3-thioxo-2-(3-cyclohexenyl)-1H-isoindol-1-one (23). The mixture of compound **21** (68 mg, 0.3 mmol) and LR (121 mg, 0.3 mmol) in toluene was refluxed under N₂ for 10 h. The solvent was removed under vacuum, and the residue was purified by column chromatography using petroleum ether as the eluent to obtain compound **22** (37 mg, 48%) as a dark green solid. Then, using CH₂Cl₂:petroleum ether (1:1) as the eluent, the more polar component **23** (23 mg, 32%) was obtained as a red solid.

Compound **22**: mp 93 °C; ¹H NMR (CDCl₃) δ 7.65–7.60 (m, 2H), 7.49–7.42 (m, 2H), 5.92–5.88 (m, 1H), 5.66–5.63 (m, 1H), 5.47–5.43 (m, 1H), 2.40–2.35 (m, 1H), 1.99–1.95 (m, 2H), 1.75–1.59 (m, 3H); MS (CI/CH₄) *m/z* 259 (M⁺); Anal. (C₁₄H₁₃NS₂) C, H, N.

Compound **23**: mp 67–68 °C; ¹H NMR (CDCl₃) δ 7.94–7.91 (m, 1H), 7.73–7.64 (m, 3H), 5.92–5.88 (m, 1H), 5.60–5.51 (m, 2H), 2.27–2.10 (m, 3H), 1.96–1.76 (m, 2H), 1.81–1.70 (m, 1H); MS (CI/CH₄) *m/z* 243 (M⁺); Anal. (C₁₄H₁₃NOS) C, H, N.

Assessment of Compounds on the Secretion of TNF-α by PBMCs. Freshly prepared PBMCs were utilized in all studies. Blood, 40 mL, was drawn from a volunteer, immediately mixed with 50 U/mL Na heparin and was diluted to 50 mL total volume with sterile PBS. Samples, 20 mL, of this preparation then was layered on 20 mL of Ficoll-Paque and was centrifuged (800*g*, 20 min). The Ficoll/serum interface, containing PBMCs, was collected, diluted to 200 mL with PBS, and then was centrifuged (800*g*, 15 min) to pellet the cells. Thereafter, the recovered pellet was resuspended in 37 °C tissue culture medium (RPMI/1 mM Sodium pyruvate/10% heat inactivated FBS/2 mM Glutamax) and placed on ice. Recovered cells were counted, pipetted (200 μL of 5 × 10⁵/mL) into 96-well plates, and incubated for 1 h (37 °C, 5% CO₂). Thereafter, appropriate concentrations of test compounds or vehicle (10 μL of DMSO) were added to duplicate wells. Following a further hour of incubation, a 10 μL sample of

lipopolysaccharide (LPS)(100 ng/mL in supplemented medium) or vehicle was added to induce stimulated and unstimulated cells, respectively, and the cells were incubated overnight. Sixteen hours later, supernatants were collected for quantification of TNF-α levels by ELISA assay (Pierce-Endogen human TNF-α minikit, Rockford, IL) and the use of specific capture and detection monoclonal antibodies, M303E and M302B (Pierce-Endogen), respectively. ELISA plates were read at 450 nm λ, and TNF-α levels were determined from a six-point calibration curve that was run concurrently with the test samples. The effect of test drug concentrations on the cellular viability of PBMCs was assessed by MTS assay (Promega, Madison, WI) of the cells that provided the supernatant samples assayed for TNF-α levels, described above.

Assessment of Compounds on TNF-α/Luciferase Activity. This cell-based assay utilized two stably transfected cell lines derived from the mouse macrophage line, RAW264.7. One line, designated “luciferase only” expressed a luciferase reporter construct without any UTR sequences. The other line, designated “luciferase + TNF-α UTR” expressed a luciferase reporter construct with the entire 3′-UTR of human TNF-α inserted directly downstream of the luciferase coding region. Compounds were added in a concentration-dependent manner, as above, and at the end of the incubation period (16 h, 37 °C, 5% CO₂) the media was removed, cells were lysed, and luciferase activity was assayed with Steady-glo luciferase assay reagent (Promega) according to the supplier's directions. Background was subtracted, and data from this assay was expressed as a ratio of the +3′-UTR to -3′-UTR (control) values and was expressed as a percent as shown in Figure 3. In this manner, compounds that show a differential effect on the two cell lines, with and without a 3′-UTR, are highlighted.

Acknowledgment. The authors wish to thank Drs. Gordon Powers and Gretchen Temeles (Message Pharmaceuticals, Malvern, PA) for the numerous assays and valued input that they each provided into this research project.

Supporting Information Available: HMBC spectrum of **5**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

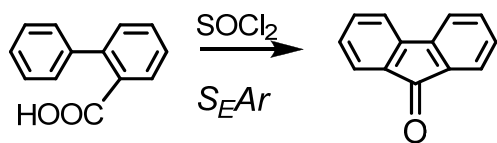
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Задача 4.

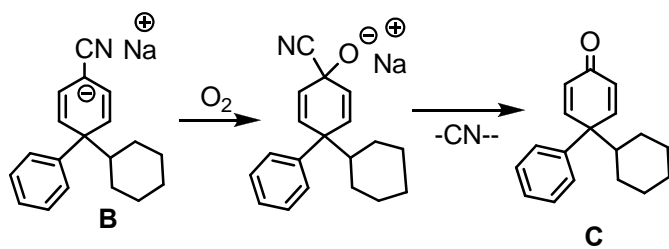


Источник - реальный студенческий эксперимент.

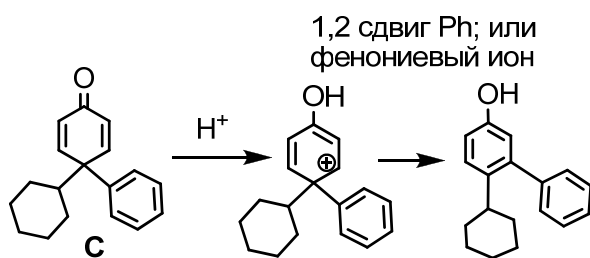
Существуют более мягкие условия, по сравнению с кипячением в хлористом тиониле, для синтеза хлорангида бифенил-2-карбоновой кислоты. (оксалил хлорид, ДМФ, CH_2Cl_2 , $0-5^\circ\text{C}$).

Matsuhisa, Akira et al. *Chemical & Pharmaceutical Bulletin*; 46; 10; 1998; 1566 - 1579

Задача 5.



через элиминирование циангидрина, среда щелочная.

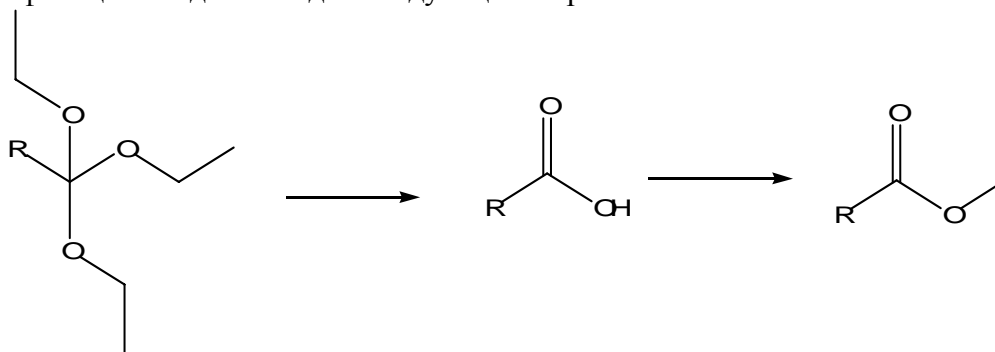


диенон-фенольная перегруппировка **D**

Дж.Дж. Ли. Именные реакции.
Механизмы органических реакции,
Бином, 2006.

Задача 6.

Изменение массы продукта относительно массы исходного эфира означает, что молекулярная масса метилового эфира в 2 раза меньше молекулярной массы этилового. Отсутствие реакции с бромной водой означает, что в исходном эфире нет кратных связей. Кроме того, отсутствие в ИК-спектре полосы поглощения в области $1550-1700\text{ см}^{-1}$ говорит о том, что в исходном эфире нет карбонильной группы. Таким образом, вещество не является сложным эфиром. Помимо сложных эфиров, существуют так называемые ортоэфиры, которые при гидролизе также дают карбоновые кислоты. Схема переэтерификации тогда выглядит следующим образом:



Здесь $R = C_xH_{2x+1}$. По спектру ПМР можно понять, что R – этильная группа. Таким образом Юный химик получил в свое распоряжение этиловый ортоэфир пропионовой кислоты. Кроме того, задачу можно решить, составив уравнение, связывающее молекулярные массы исходного и конечного эфиров:

$$12 \cdot x + 2x + 1 + 147 = 2 \cdot (12x + 2x + 1 + 59)$$

$$14 \cdot x + 148 = 28 \cdot x + 120$$

$$14 \cdot x = 28$$

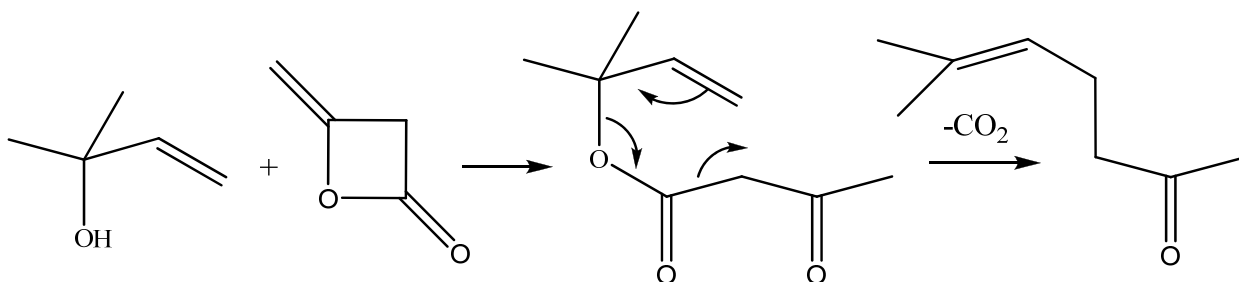
$$x = 2, \text{ т.о. } R = C_2H_5$$

P. S. Автор не исключает и другого решения задачи, однако ни одним из участников олимпиады оно не было предложено.

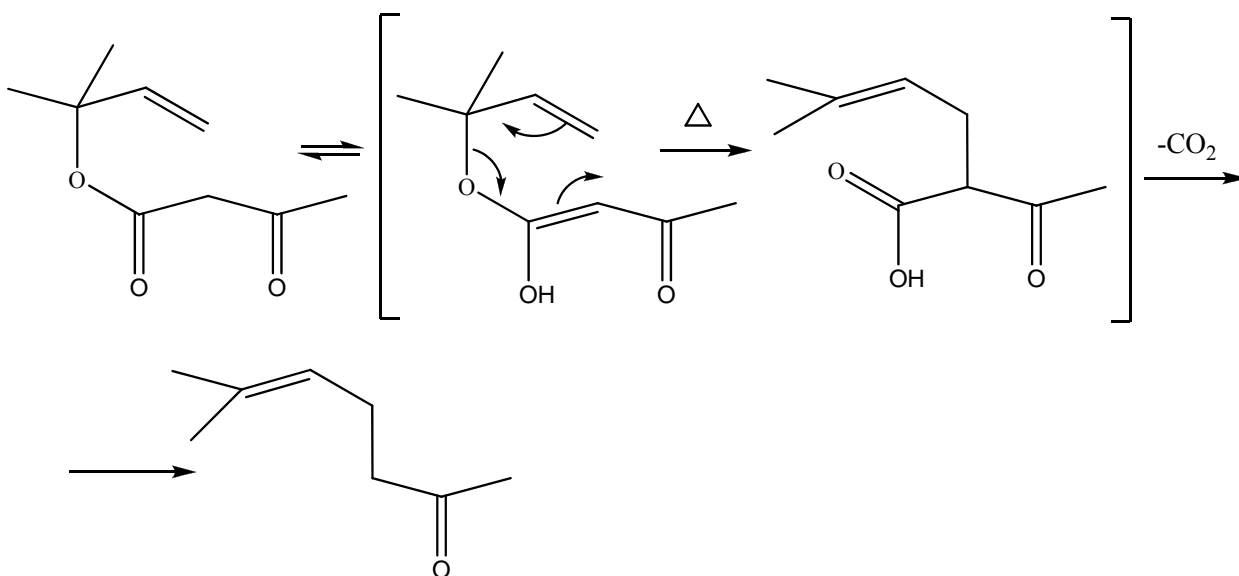
Задача 7.

К сожалению, решений может быть несколько в зависимости от степени подробности (например в п. 1) или в последовательности стадий (в пункте 2, в данном случае приведено оригинальное, наиболее рациональное решение)

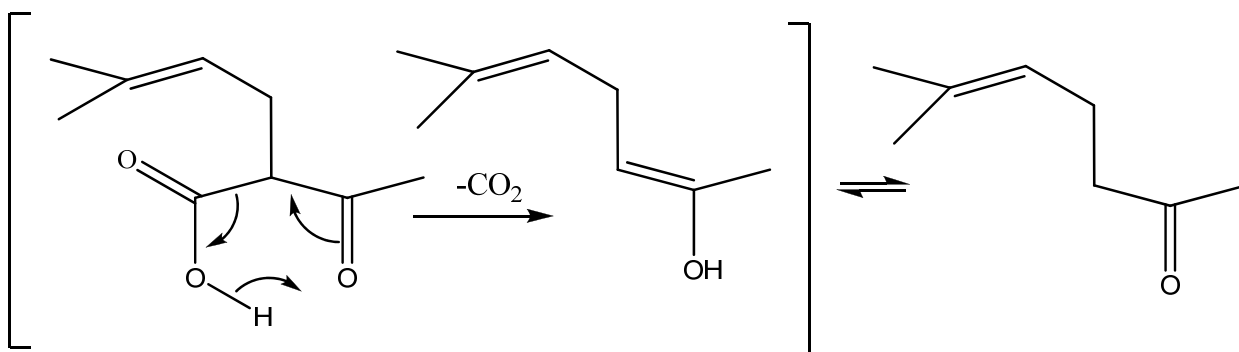
1) Кратко:



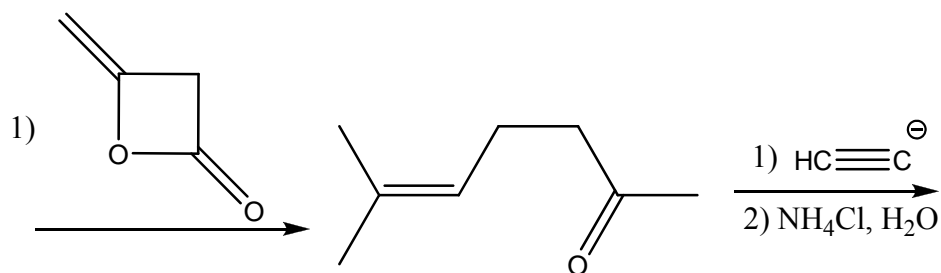
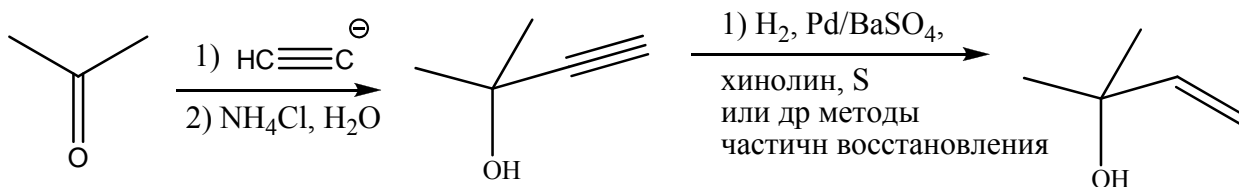
Чуть более подробно:



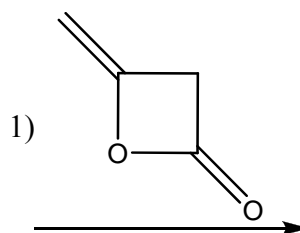
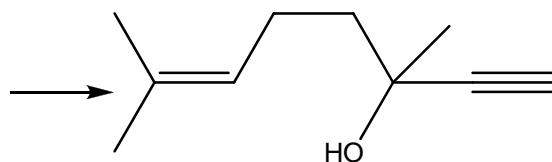
Или даже:



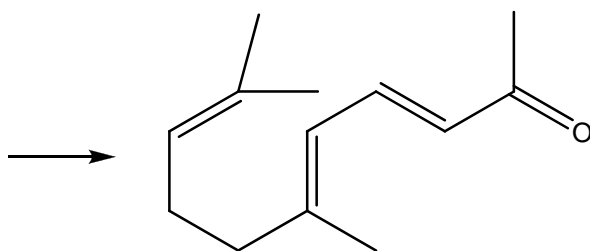
2)



2) Нагревание

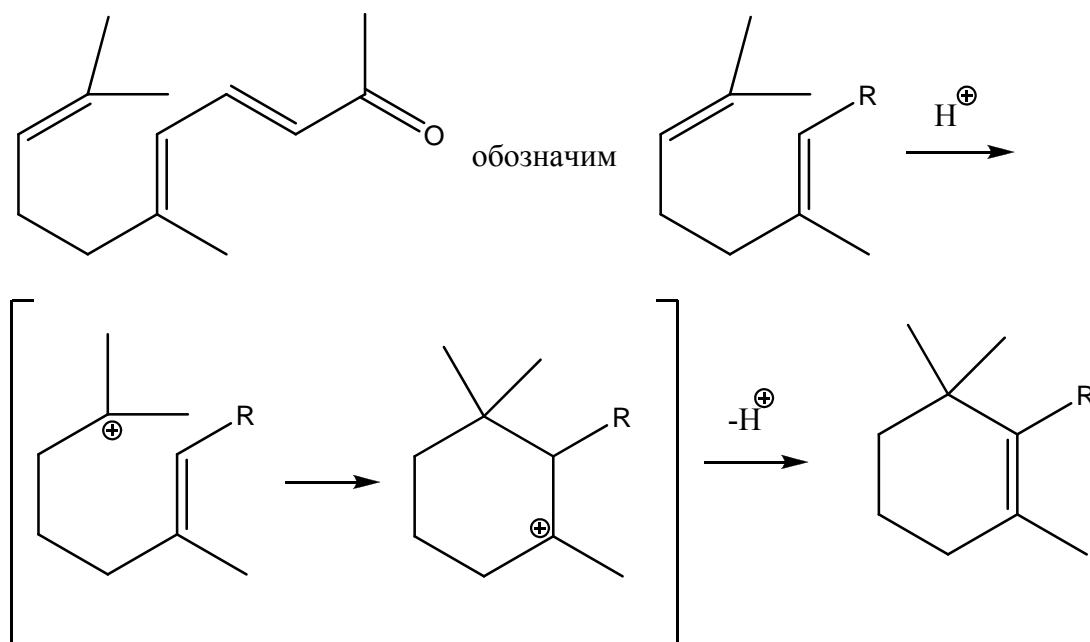


2) Нагревание

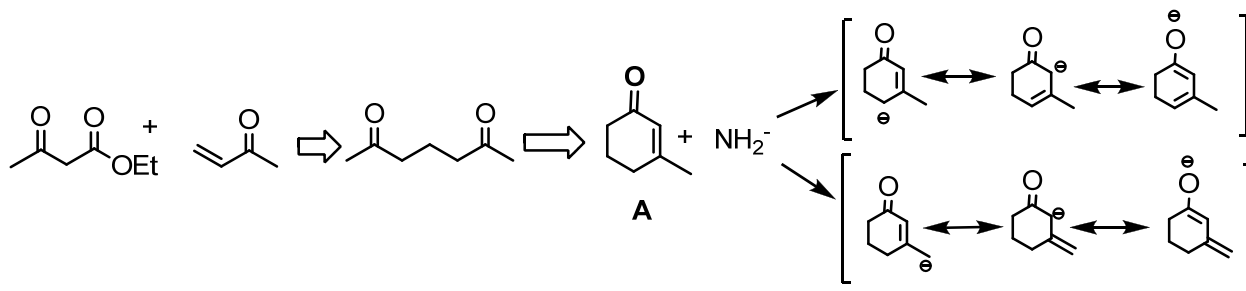


Действуя по аналогии, сначала можно было бы частично восстановить тройную связь, затем провести реакцию с дикетеном и в продукте перегруппировки создать недостающую двойную связь, данный метод не был бы ошибочным, только более длинным и менее рациональным. К счастью, подобная перегруппировка возможна для пропаргилового спирта также как и для аллилового.

3) Здесь возможен следующий вариант:



Задача 8.



Керри Ф., Сандберг Р. Углубленный курс органической химии. М.: Химия, 1981.

Задача 9.

