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Facile Synthesis of an Azido-Labeled Thalidomide Analogue

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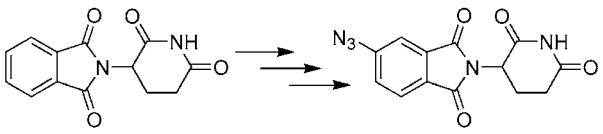
ABSTRACT



A five-step synthesis of an azido-thalidomide analogue is presented. The sequence requires cheap and readily available starting materials and reagents, and only two steps require purification. Additionally, the azido-labeled analogue possesses activity comparable to that of thalidomide in inhibiting the proliferation of human microvascular endothelial cells, thus providing impetus for its use as a potential photoaffinity label of thalidomide.

Thalidomide was developed in the 1950s by Chemie Grünenthal of Germany as a nontoxic sedative.[1] It was widely used to prevent morning sickness in pregnant women. In addition to its sedative effects in humans, an association was reported of teratogenic limb defects from maternal thalidomide usage.[1] Aside from this serious teratogenic effect on the fetus, the drug does have therapeutic value: (1) for its immunosuppressive effect in the treatment of graft versus host disease;[2] (2) in the treatment of leprosy;[3] and (3) for inflammatory dermatoses.[4] Furthermore, thalidomide has significant antiangiogenic activity[5] and, as a result, is finding more extensive use in the treatment of various cancers in which poor prognosis is dependent upon microvessel density such as multiple myeloma,[6] colon,[7] prostate,[7] and breast[8] cancers.

The drug is marketed as a racemic mixture, although it has been suggested that the sedative hypnotic effect is associated with the (*R*)-isomer and the teratogenic effects are associated with the (*S*)-isomer (Figure 1).[9] Recent reports

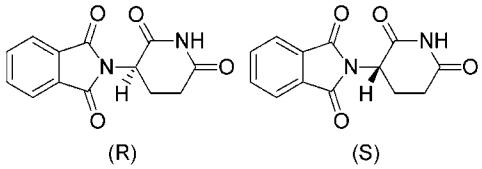


Figure 1. Structures of (*R*)- and (*S*)-thalidomide.

show that, at physiological conditions, the strongly acidic hydrogen atom at the asymmetric center of thalidomide rapidly epimerizes, rendering biossay of enantiomers difficult.[10] Furthermore, the exact mechanism of thalidomide teratogenesis still remains unknown.

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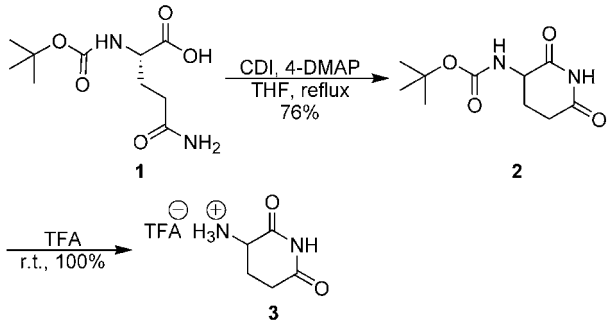
Thus, it is our goal to undertake a synthetic and biological strategy to provide insight into the mechanism of action of thalidomide and related analogues. We propose the synthetic aspect of the strategy to include the synthesis of an azido analogue of thalidomide, which will then be examined biologically for its potential use as a photoaffinity label. The use of photoaffinity analogues to assist in elucidating mechanisms of action has been reported in the literature.[11] To our knowledge, there is only one other example of a thalidomide photoaffinity label reported in the literature,[12] but the present analogue more closely resembles the actual structure of thalidomide.

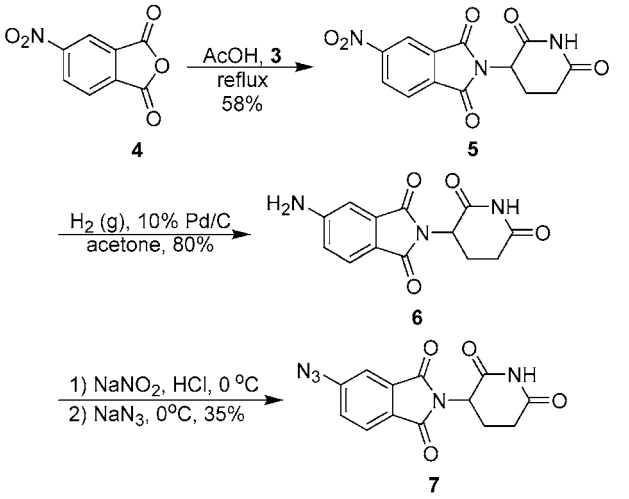
The synthesis of the azido analogue is broken down into two parts. The first involves the synthesis of the right half of the compound, or the glutarimide moiety (Scheme 1).

Scheme 1. Synthesis of the Glutarimide Moiety



The synthesis of **3** begins by utilizing the method of Muller et al.[13] in the treatment of the commercially available *N*α-(*tert*-butoxycarbonyl)-L-glutamine **1** with 1,1′-carbonyldiimidazole (CDI) and 4-dimethylamino pyridine in refluxing THF to afford **2**, in which racemization of the product occurs.[14] The resultant *N*-Boc glutarimide ring **2** is subsequently deprotected by TFA at room temperature to afford to the glutarimide moiety **3** as the TFA salt. Attempts to isolate the free amine resulted in rapid decomposition, possibly by hydrolysis of the glutarimide ring. Once in hand, intermediate **3** was condensed with 4-nitrophthalic anhydride **4** by refluxing in glacial acetic acid over 2−3 h, affording the 4-nitro-substituted thalidomide derivative **5**[15] in 58% yield (Scheme 2).

Scheme 2. Synthesis of Azido Photoaffinity Analogue



Reduction of the nitro group with 10% Pd/C and H_2 (g) proceeded smoothly to yield the 4-amino-substituted analogue **6**[15] in 80% yield. Diazotization of **6**, by treatment with sodium nitrite in aqueous HCl at 0 °C, followed by the slow addition of sodium azide, afforded the 4-azido-substituted thalidomide analogue **7** in 35% yield. It is important to note that the reaction conditions are not yet optimized for this series of transformations; thus, it is believed that the moderate yields obtained for some of the steps can be improved upon.

As shown in Table 1, the azido-labeled analogue possesses greater potency than thalidomide in inhibiting human microvessel endothelial cell (HMEC) proliferation, both in the presence and absence of vascular endothelial growth factor (VEGF).[16] Additionally, the endothelial cell proliferation effect exhibited by **7** proves that the addition of the azido group to thalidomide is not detrimental to the compound's ability to interact with the thalidomide binding site(s).

Table 1. Inhibition of HMEC Proliferation by Analogue **7**

	HMEC IC_{50} (μM)[a]	
compound	(+) VEGF	(−) VEGF
thalidomide	>300	>300
7	259 ± 31.2	239 ± 55.8

[a] IC_{50} values reported are averages of triplicate experiments.

Herein, we have described a facile synthesis of a novel azido photoaffinity thalidomide analogue. The teratogenic effects of the parent thalidomide are well-known throughout the chemical and biological community, although the mechanism by which the drug elicits these effects remains unknown. The fact that the (*S*)-enantiomer of thalidomide exhibits the strongest antiangiogenic and teratogenic activity[17] shows that there is a clear enantioselective preference, and

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(14) For **1**: $[\alpha]_D$ −10.4 (*c* 0.1, CH_3OH). For **2**: $[\alpha]_D$ 0.0 (*c* 0.1, CH_3OH).

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(16) VEGF is a known stimulator of endothelial cell proliferation.

this preference, coupled with structure activity relationships,[18] lends support to a possible receptor-mediated mechanism-(s).

We anticipate that this new azido-labeled analogue of thalidomide will be valuable in aiding in the identification of relevant protein interactions and in elucidating putative binding site(s). The compound has already proven to be comparable to thalidomide in the inhibition of HMEC cell proliferation (Table 1). Future work will include coupling our azido analogue to selected protein targets to examine its potential use as a photoaffinity label.

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Supporting Information Available: Synthetic and biological experimental procedures and full characterization of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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