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Synthesis of novel glutarimide derivatives *via* the Ugi multicomponent reaction: affinity towards the E3 ubiquitin ligase substrate receptor Cereblon

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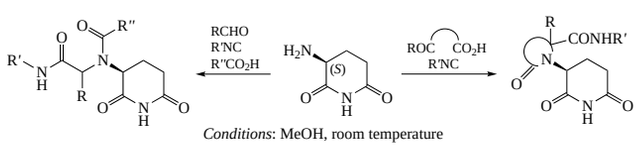
The glutarimide moiety, common in many immunomodulatory drugs, was decorated with lactam and diamide side chains *via* two variants of the Ugi reaction, namely, with isocyanide, aldehyde and acid or with isocyanide and oxo acid. The resulting diastereomerically pure compounds were evaluated for their affinity towards the E3 ubiquitin ligase substrate receptor Cereblon.



Keywords: 2-aminoglutarimide, Ugi reaction, oxo acids, isocyanides, immunomodulatory drugs, E3 ubiquitin ligase, Cereblon.

The immunomodulatory imide drugs (IMiDs) thalidomide, lenalidomide and pomalidomide are all approved for the treatment of multiple myeloma [Figure 1(*a*)].[1] The mechanism of action of these drugs includes binding to Cereblon (CRBN), substrate receptor subunit of the E3 ubiquitin ligase complex $CRL4^{CRBN}$.[2] Binding of IMiDs to CRBN initiates ubiquitination of non-endogenous substrates such as the transcription factors IKZF1/3, which leads to their proteasomal degradation and growth inhibition in multiple myeloma cells.[3] The binding of IMiDs to CRBN also forms the basis for the so-called proteolysis

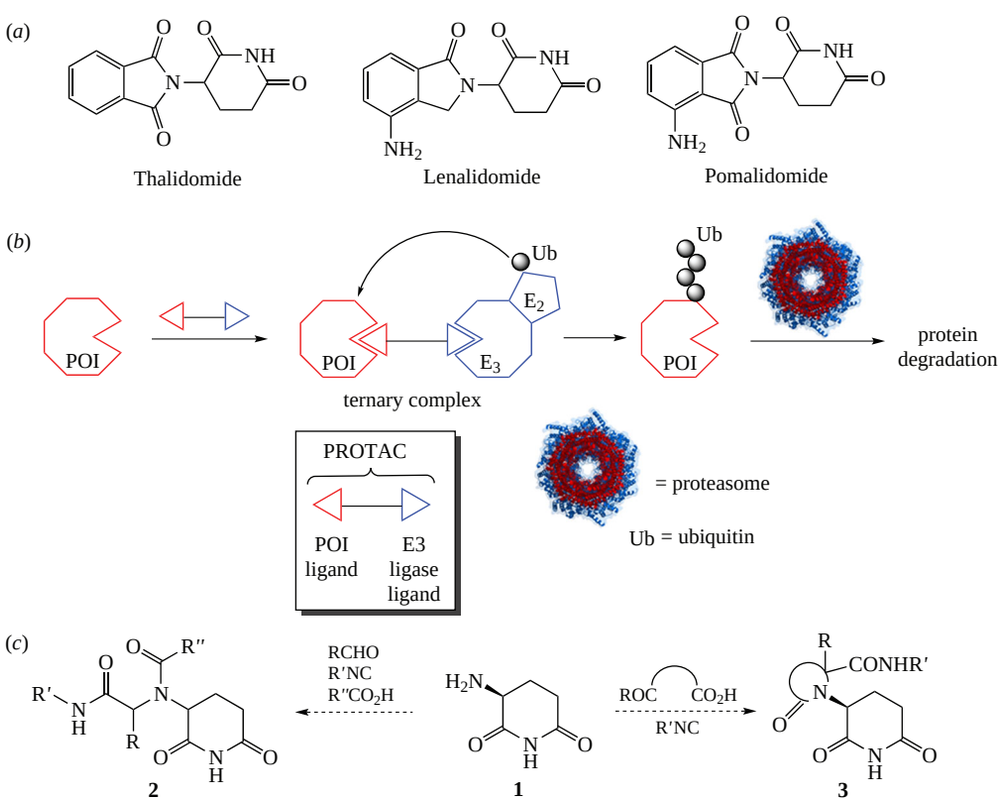
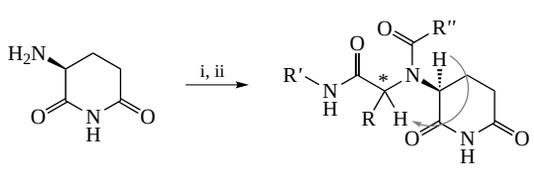


Figure 1 (*a*) Structures of IMiDs in clinical use; (*b*) principle of PROTAC functioning; (*c*) glutarimide derivatives explored in this work.

targeting chimeras (PROTACs), heterobifunctional constructs comprising ligands for a protein of interest (POI) and an E3 ubiquitin ligase connected by a linker.[4] PROTACs help to bring the POI and a E3 ligase, here $CRL4^{CRBN}$, together to form a ternary complex. This promotes ubiquitination and ultimately proteolytic degradation of the POI by the proteasome [Figure 1(*b*)].[5] This approach, commonly referred to as targeted protein degradation is a highly promising alternative to the use of small molecule inhibitors and other approaches which lower aberrant protein levels or activity.[6] Thalidomide and its derivatives are notoriously associated with potential teratogenic effects, which motivates the current extensive search for alternative CRBN ligands.[7–9] While the glutarimide portion of IMiDs is essential for CRBN binding, the search is focused on diversely substituted glutarimides[10] starting from various glutarimide building blocks.[11] (*S*)-2-Aminoglutarimide [(*S*)-3-aminopiperidine-2,6-dione] **1** is readily available, hence, we considered employing it as the amine component for the Ugi multicomponent reaction.[12] Besides the traditional four-component format of the latter leading to diamides of type **2**, we were specifically interested in the three-component four-center (3C4C) Ugi reaction of oxo carboxylic acids[13] leading to lactam derivatives of glutarimide **3** which are structurally related to IMiDs [Figure 1(*c*)]. Herein, we report on the realization of this synthetic strategy.

Non-racemic starting (*S*)-2-aminoglutarimide **1** was synthesized in multigram scale in nearly quantitative yield over two steps from *N*-Boc-protected L-glutamine as described.[14] It was reacted with *p*-tolualdehyde in methanol (over 30 min), whereupon *tert*-butyl or cyclohexyl isocyanides and acetic or isobutyric acids were added, and the reaction proceeded to completion in 24 h (Scheme 1).[15] The resulting products **2a–c** were formed as mixtures of diastereomers which were found to be easily separable by conventional column chromatography on silica gel. The individual diastereomers were obtained in modest yields and their stereochemistry was tentatively assigned (see the gray arrow for observed NOESY correlations). Notably, the major, more polar diastereomer, to which the *R*,*S*-configuration was assigned (see Online Supplementary Materials), was obtained in 2–3-fold higher yield compared to the minor *S*,*S*-diastereomer (see Scheme 1). The combined yield of compounds **2a**,**b** was good to excellent; however, the use of bulkier isobutyric acid lowered the yield of the corresponding adduct **2c**.

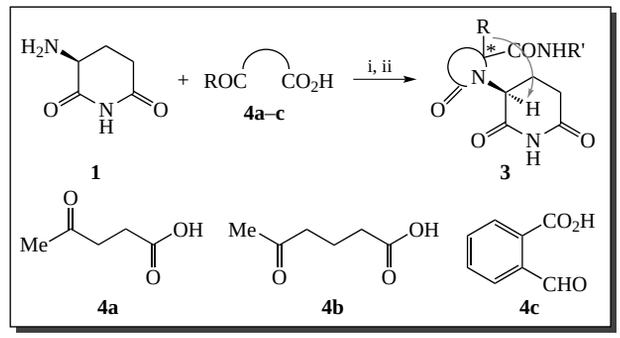
Under identical conditions, (*S*)-2-aminoglutarimide **1** reacted with 4-oxopentanoic acid **4a**, 5-oxohexanoic acid **4b** or *o*-formylbenzoic acid **4c** and *tert*-butyl or cyclohexyl isocyanides (Scheme 2). The resulting lactams **3a–d** were also obtained as mixtures of diastereomers separable by column chromatography; the stereochemistry (see Online Supplementary Materials) was tentatively assigned based on NOESY correlational spectra

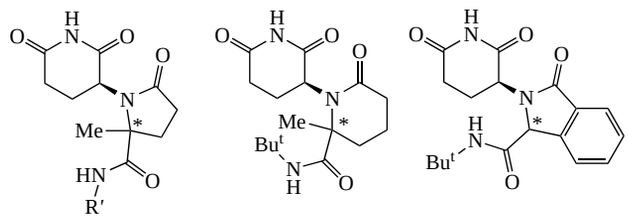


1 **2a–c** (R = *p*-Tol)

a R′ = Bu^t, R″ = Me
(*S*,*S*) 30%, (*R*,*S*) 62%
b R′ = Cy, R″ = Me
(*S*,*S*) 18%, (*R*,*S*) 56%
c R′ = Bu^t, R″ = Pr^i
(*S*,*S*) 8%, (*R*,*S*) 30%

Scheme 1 *Reagents and conditions*: i, 4-MeC_6H_4CHO, MeOH, room temperature, 30 min; ii, Bu^tNC or CyNC and AcOH or Pr^iCO_2H, room temperature, 24 h.





3a,**b** **3c** **3d**
(*S*,*S*) 17%, (*R*,*S*) 9% (*S*,*S*) 11%, (*R*,*S*) 7%

a R′ = Bu^t
(*S*,*S*) 42%, (*R*,*S*) 45%
b R′ = Cy
(*S*,*S*) 49%, (*R*,*S*) 32%

Scheme 2 *Reagents and conditions*: i, MeOH, room temperature, 30 min; ii, RNC, room temperature, 24 h.

(correlations observed are denoted by the gray arrow). It is notable that the four-center three-component Ugi reaction worked exceptionally well for 4-oxopentanoic acid **4a** and afforded 89 and 81% combined yields of lactam adducts **3a** and **3b**, respectively, after chromatographic separation of the individual diastereomers. In contrast, the same reaction of homologous 5-oxohexanoic acid **4b** gave only a 26% combined yield of product **3c**. Similarly, poor reaction outcome was observed for *o*-formylbenzoic acid **4c**, with the combined yield of lenalidomide analogue **3d** of only 18%. All attempts to improve the yields of adducts **3c**,**d** by increasing reaction time, raising the reaction temperature or altering the reagent ratios were not successful.

All the new *NH*-glutarimide compounds **2a–c** and **3a–d** were tested for their affinity to the thalidomide-binding domain of human CRBN (*h*TBD) (Table 1) using the recently developed microscale thermophoresis assay, which measures the outcompeting of a fluorescent reporter ligand by the compound.[16]

To our delight, although CRBN affinity displayed by new compounds **2a–c** and **3a–d** was generally lower than that of thalidomide, these compounds showed confident binding to CRBN in the tens to hundreds micromole per liter range. In the context of future design of PROTAC-type constructs, high affinity to neither the POI nor the E3 ubiquitin ligase is needed,

Table 1 Affinity of individual diastereomers of compounds **2a–c** and **3a–d** to the thalidomide-binding domain of human CRBN. *N* = 3 for (*R*,*S*)-**2a**, (*R*,*S*)-**2b** and **1** with ±95% CI, *N* = 1 for all other compounds.

Compound	CRBN affinity (IC_{50}/μM)	Compound	CRBN affinity (IC_{50}/μM)
1	22.9 ± 1.6	(*S*,*S*)-**3a**	256
(*S*,*S*)-**2a**	267	(*R*,*S*)-**3a**	394
(*R*,*S*)-**2a**	54.7 ± 11.9	(*S*,*S*)-**3b**	298
(*S*,*S*)-**2b**	142	(*R*,*S*)-**3b**	>1000
(*R*,*S*)-**2b**	39.8 ± 5.8	(*S*,*S*)-**3c**	160
(*S*,*S*)-**2c**	118	(*R*,*S*)-**3c**	228
(*R*,*S*)-**2c**	95.9	(*S*,*S*)-**3d**	397
		(*R*,*S*)-**3d**	>1000

considering the catalytic mechanism of action of such degraders (*i.e.*, the latter should be able to enter many cycles of the ternary complex formation and disintegration, which requires moderate affinity to both proteins).[17] Unexpectedly, despite their greater structural similarity to IMiDs, lactam adducts **3a**–**d** generally displayed lower affinity to CRBN compared to open-chain diamide adducts **2a**–**c**. In the latter series, the major (*R*,*S*)-diastereomer in each pair displayed markedly (up to 5 times) tighter binding to CRBN compared to the minor (*S*,*S*)-diastereomer. In the lactam series, however, the trend was reversed with (*R*,*S*)-diastereomer being less affine to CRBN. The sensitivity of CRBN binding to the relative stereochemistry should also be noted. Indeed, while one of the diastereomers (*S*,*S*) showed a confident binding to CRBN in the 300–400 μM range for lactams **3b** and **3d**, the other diastereomer (*R*,*S*) was virtually devoid of CRBN affinity.

In conclusion, we described a novel derivatization strategy for the medicinally important glutarimide moiety *via* the traditional four-component Ugi reaction (delivering diamides) as well as the lactam-forming four-center three-component variant of the reaction involving oxo carboxylic acids. The reaction produced two diastereomers in all cases, with a preference for one of the two in the case of the open-chain diamide analogues. For every compound, diastereomers were separated chromatographically and were profiled for affinity towards the thalidomide-binding domain of human CRBN. Although the new derivatives proved less affine to CRBN compared to thalidomide (with open-chain diamide derivatives binding more tightly than their lactam counterparts), the observed binding characteristics may be sufficient for employing these CRBN ligands in the design of PROTAC-type degraders.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.11.013.

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