Synlett M. T. Scerba et al. Letter

Thionation of Aminophthalimide Hindered Carbonyl Groups and Application to the Synthesis of 3,6′-Dithionated Pomalidomides

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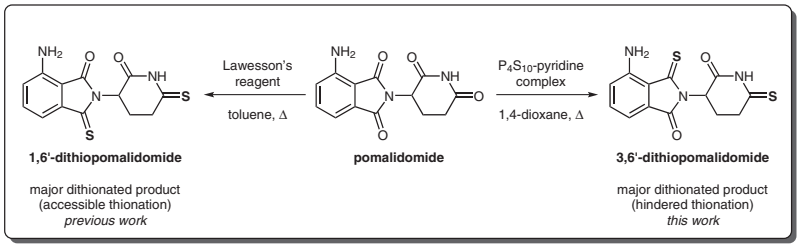

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Abstract Herein, we present a new one-pot procedure for the 3,6′-dithionation of pomalidomide derivatives in which the key 3-position sulfur atom is preferentially installed at the desired (but sterically congested) carbonyl of the aminophthalimide system and with regiochemistry distinct from Lawesson's Reagent thionation methods. When heated in 1,4-dioxane with P_4S_{10}–pyridine complex, pomalidomides are smoothly and reproducibly converted into their 3,6′-dithionated analogues in roughly 30% isolated yield and at various scales. While detrimental to the desired 3,6′-type outcome when employing Lawesson's Reagent, we hypothesize that the pomalidomide aniline group instead facilitates P_4S_{10}-type thionation at the otherwise hindered 3-position carbonyl, contributing to the selectivity observed. When paired with classical methods of thionation, this approach offers an interesting and appealing addition to the synthetic toolbox, permitting facile late-stage access to complementary thionated pomalidomides in direct single-flask procedures.

Key words thionation, pomalidomides, dithionation, aminophthalimides, regioselectivity

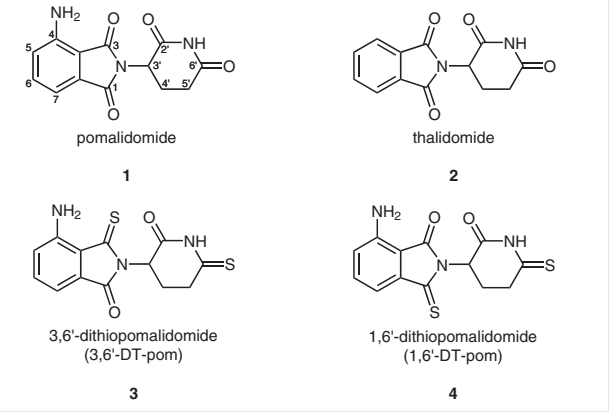


Figure 1 Structures of pomalidomide, thalidomide, and dithionated pomalidomides. The numbering pattern illustrates the locations of interest, including the 3-, 1- and 6′-position carbonyls of pomalidomide and related aminophthalimides.

Pomalidomide (**1**) is a well-known derivative of thalidomide, possessing a variety of immunoregulatory and tumoricidal properties. In this regard, it is currently being marketed for the treatment of multiple myeloma.[1] Research in our laboratory has demonstrated advantageous consequences of thionation on the efficacy and toxicity profiles of various thalidomide analogues in the treatment of mild traumatic brain injuries and other proinflammatory processes.[2] We recently identified 3,6′-dithiopomalidomide (**3**; 3,6′-DT-pom) (Figure 1) as showing particular promise in lowering classical proinflammatory markers in response to applied stress.[3] In these studies, 3,6′-DT-pom (**3**) demonstrated a similar anti-TNF-α activity to that of pomalidomide in both RAW 264.7 cellular studies and in rodents challenged with lipopolysaccharide, but additionally lowered inflammation-induced COX-2 and iNOS.

Despite these advantages, our laboratory had yet to develop even a modestly scalable method yielding 3,6′-DT-pom and similar desirable materials. Our initial syntheses (which combined pomalidomide with Lawesson's Reagent (LR) in refluxing toluene) were impeded by solubility limitations and also generated multiple thionated isomers and byproducts which were difficult to separate using standard purification methods.[4] The major dithionated product in these cases proved to be the isomeric 1,6′-dithiopomalidomide (**4**; 1,6′-DT-pom). Further compounding the issue, only minor amounts of the desired 3,6′-DT-pom were efficiently isolable from these complex reaction mixtures. Even in the best circumstances, the reactions were erratic and not reliably repeatable owing to the aforementioned limita-

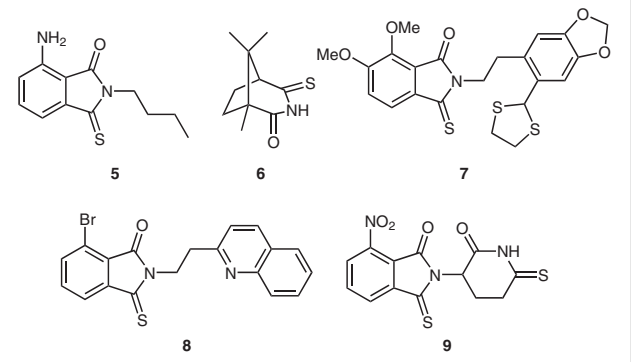


Figure 2 Thionated cyclic imides generated using Lawesson's Reagent and showing thionation at the less-hindered carbonyl

tions. Needless to say, quantities of the valuable 3,6′-DT-pom sufficient for advanced biological studies were not feasible using the Lawesson's-based method. These shortcomings, combined with our desire to investigate complementary 3,6′-type materials, led us to explore new reagents and conditions for the scalable production of 3,6′-DT-pom (**3**) and similarly thionated aminophthalimides.

Our experience with thalidomide reactivity patterns[5] led us to reason that the pomalidomide glutarimide 6′-position would be easily thionated with almost any of the reagents we would consider for our new method. Therefore, the difficult transformation (and the main hurdle needed to be cleared) would be preferential thionation at the sterically encumbered 3-position carbonyl concurrent with abated reactivity at the readily available 1-position. Previous studies on Lawesson's-type thionations of cyclic imides demonstrated that the reactions occur preferentially at the less crowded carbonyl, and are largely independent of simple electronic effects (Figure 2). In these regards, -NH_2, -CH_3, -Br, -OMe, and -NO_2 substitution proximal to a carbonyl can direct thionation to the opposing side of the imide, suggesting that a new paradigm could be required to achieve preferential reactivity at our desired positions in pomalidomide.

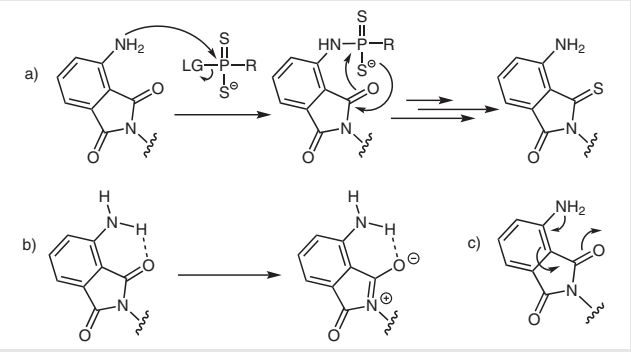
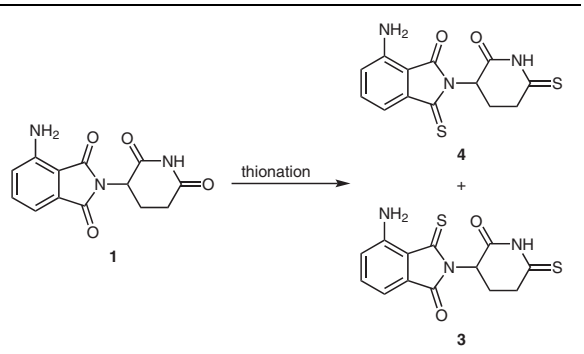


Figure 3 Hypothetical reaction pathways for thionation of the pomalidomide aminophthalimide moiety with a focus on the desired 3-position carbonyl. (a) Interaction of the 4-position amino group with a sterically accessible phosphorus-based thionating agent. The thionating center locates in proximity to the desired 3-position carbonyl. (b) Distorted six-membered hydrogen bonding. (c) Increasing local carbonyl electron density via resonance/inductive effects. LG = leaving group.

While seemingly detrimental to Lawesson's-type reactivity at the pomalidomide 3-position carbonyl, we hypothesized that the phthalimide 4-amino group might instead be utilized to our advantage if combined in tandem with the proper thionation partner. We initially envisioned the most direct use of this aniline could be as a molecular chaperone, guiding an appropriately compact non-Lawesson's-type reagent toward the desired 3-position (Figure 3a). Presumably, a thionation center substituted by an appropriate leaving group could permit amine attack, thereby localizing the sulfur-transferring source proximal to the object carbonyl. Alternatively, the aniline could facilitate the desired thionation via hydrogen bonding[7] or through inductive/resonance electronic effects (Figure 3b,c).[8] In this regard, a variety of conceivable pathways unfavored with Lawesson's Reagent or simply unavailable to the 1-position carbonyl could prime the 3-position towards thionation, and especially when conditions might otherwise and independently support a more accessible location.

Table 1 Screen of Pomalidomide Thionation Conditions Highlighting the Multithionated Product Distributions



Entry	Reagent	Solvent	Time (h)	Ratio **4**/**3**[a]
1	LR	toluene	18 h	4:1[b]
2	P_4S_{10}–pyr	pyridine	2 h	1:10[b]
3	P_4S_{10}–pyr	MeCN	2 h	**3** only[b]
4	P_4S_{10}–pyr	1,4-dioxane	2 h	**3** only[c]
5	P_4S_{10}	1,4-dioxane	2 h	**3** detected with indication of trithionated materials
6	P_4S_{10} + py	1,4-dioxane	2 h	1:44[b]

[a] Approximated using LC/MS extrapolated peak areas.
[b] Reaction not fully complete; starting pomalidomide material and/or monothionated intermediates remain clearly detectable.
[c] The desired material **3** is the overwhelmingly major peak by far; starting or monothionated materials are trace components.

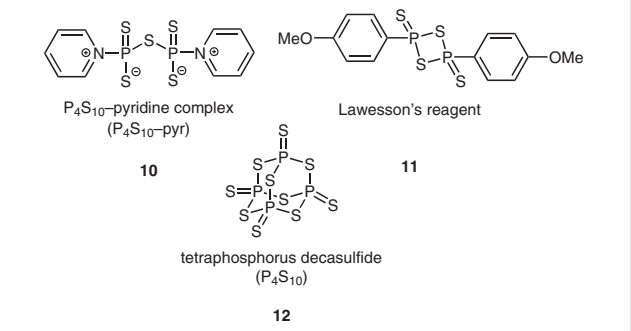
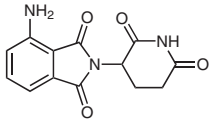
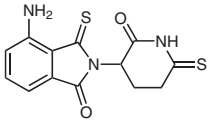
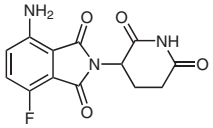
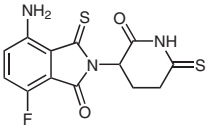
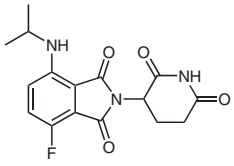
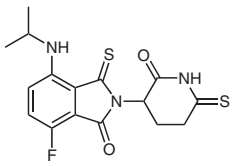
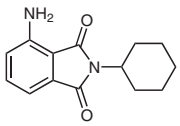
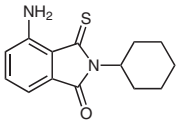


Figure 4 Structures of relevant thionating reagents

When searching for appropriate reagents, we noticed the recently investigated P_4S_{10}–pyridine complex **10** (P_4S_{10}–pyr; Figure 4).[9] It offered many desirable traits compared with LR, including ease of preparation, simplified workup, amenability to a variety of solvents, and, most interestingly, a potential leaving group in the form of a pyridinium ligand.

Initially, we screened P_4S_{10}–pyr with pomalidomide in pyridine, acetonitrile, or 1,4-dioxane in a series of LC/MS experiments and were pleased to discover 3,6′-DT-pom as the major dithionated isomer, with only trace amounts of undesired 1,6′-DT-pom detected in certain cases (Table 1).[10] The reaction was comparatively sluggish in pyridine, with significant amounts of pomalidomide starting material and presumed monothionated intermediates present after two hours. A very minor amount of the 1,6′-isomer was present as well. During the same timeframe, the reactions in both acetonitrile and dioxane were absent of detectable 1,6-DT-pom, with the MeCN iteration showing minimal remaining monothionated adducts, while the dioxane attempt was driven essentially to completion. Given the structural similarities between P_2S_5 (generated during dissociation of **12**) and the P_4S_{10}–pyr complex **10**, we wondered if traditional P_4S_{10} would be a viable thionation reagent in our system.[11] When pomalidomide was screened with P_4S_{10} in 1,4-dioxane, we noticed a rapid consumption of the starting material and significant production of 3,6′-DT-pom. Unfortunately, the reaction was quite indiscriminate, being complicated by the production of 1,6′-DT-pom and materials consistent with higher trithionated masses[12] even after 15 minutes; by two hours of reaction time, it was clear that the reaction was being driven towards the trithionated congeners.[13] In an attempt to attenuate this harsh reactivity, we performed

Table 2 Regioselective Mono- and Dithionation of Aminophthalimides with Associated Yields[a]

Entry	Substrate	Product	Scale	Yield, Avg.[b]
1	 **1**	 **3**	25 mg (0.09 mmol) 100 mg (0.36 mmol) 274 mg (1 mmol)	8.0 mg (29%) 35 mg (31%) 96 mg (31%)
2	 **13**	 **14**	25 mg (0.09 mmol)	8.6 mg (29%)
3	 **15**	 **16**	30 mg (0.09 mmol)[c]	12 mg (36%)
4	 **17**	 **18**	89 mg (0.36 mmol)[d] 244 mg (1 mmol)	45 mg (47%) 178 mg (68%)

[a] Reaction conditions: pomalidomide (1.0 equiv), P_4S_{10}–pyr (1.5 equiv), 1,4-dioxane, 100 °C, under N_2. The reaction was monitored by LC/MS, and the product was purified by chromatography.
[b] Average yield of two or three iterations, see Supporting Information.
[c] P_4S_{10}–pyr (2 equiv), 40 h.
[d] P_4S_{10}–pyr (0.75 equiv) was used (based upon the carbonyl stoichiometry relative to pomalidomide).

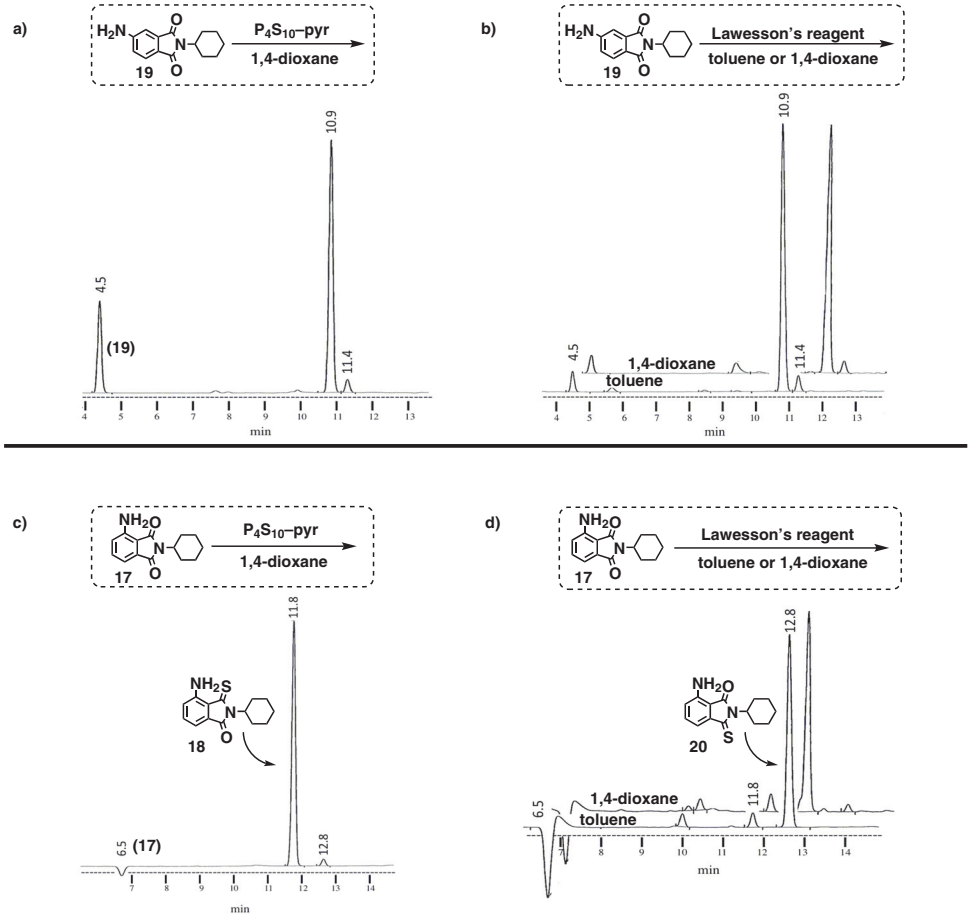


Figure 5 Representative HPLC traces demonstrating amine electronic influence on reactivity patterns of isomeric aminophthalimides with differing thionation systems at 1 h of reaction time. Materials were thionated with either P_4S_{10}–pyridine complex or Lawesson's Reagent and using the indicated solvent (1,4-dioxane or toluene) (see the Supporting Information for complete details). In systems (a) and (b), starting material **19** elutes at 4.5 min, monothionated adducts appear at 10.9 min and 11.4 min (inferred by mass). In these instances, the regiochemical differences between thionation protocols are seemingly negligible. In systems (c) and (d), starting material **17** elutes at 6.5 min, and the regiochemical bias is clear, with the indicated major monothionated species eluting at 11.8 min (c) and at 12.8 min (d) (toluene or 1,4-dioxane). Spectra were obtained at 280 nm.

a similar reaction but with added pyridine to crudely approximate the *in situ* production of the P_4S_{10}–pyr reagent.[14] We were cautiously optimistic to find that this iteration proceeded in a much more controlled fashion, especially given the sensitivity of multicomponent P_4S_{10}-derived thionations to reagent quality, reaction conditions, and the choice of the imide-type substrate.[15] Whereas this method was clearly superior to using P_4S_{10} alone, there was still a noticeable amount of undesired 1,6′-DT-pom (**4**) produced in addition to other thionated isomers. Ultimately, the P_4S_{10}–pyridine complex **10**/1,4-dioxane motif afforded a much cleaner LC/MS profile in our screen, demonstrated consistent reproducibility, and was selected as the method of choice for all further synthetic reactions.

Next, a small family of aminophthalimides reacted smoothly in one-pot procedures to produce the desired thionated pomalidomide derivatives in moderate but consistent yields (Table 2).[16] Most importantly, the relevant 3-position thionations occurred cleanly and without significant competition from the 1-position carbonyl. Pomalidomide (**1**) afforded the key 3,6′-dithiopomalidomide (**3**) in 29% isolated yield; this reaction worked equally well at all scales examined. Halogen substitution *para* to the amine appeared to have little effect on the reactivity pattern, as fluorinated analogue **14** was generated in 29% yield. Notably, the bulky *N*-isopropyl analogue **15** provided hindered **16** as the major dithionated product in 36% isolated yield. Finally, cyclohexyl-substituted analogue **18** was readily obtained, particularly at the 1 mmol scale.

To further probe this reactivity and highlight the regiochemical preference for the 3-position carbonyl, we subjected isomeric cyclohexyl aminophthalimides **17** and **19** to a series of thionation conditions (Figure 5). In substituting the glutarimide ring of the pomalidomide backbone with a simple cyclohexane, we simplified our subsequent analyses by eliminating any possible thionation event at the uninter-

esting 6′-position, while simultaneously relieving some of the potential electronic influence and steric congestion neighboring the key imide moiety. With the amine removed from direct proximity to the 3-position carbonyl, thionations of 5-aminophthalimide **19** using P_4S_{10}–pyridine or LR (Figure 5a,b) yielded nearly identical HPLC profiles after a one hour reaction time, with two resulting monothionated products[12] generated in practically equal ratios and largely independent of the thionation reagent or solvent employed (Figure 5; product peaks 10.9 and 11.4 min). With the adjacent amine reestablished in 4-aminophthalimide **17**, the expected regiochemical preferences resurfaced, with LR providing a distinct major 1-thionated species **20** (12.8 min) versus the 3-thionated product **18** (11.8 min) obtained with the P_4S_{10}–pyr/dioxane system (Figure 5d,c). It is evident that in this simplified system, any inherent electronic activation[17] afforded by the amine alone is not the sole driving force behind the observed regiochemical bias; if this were the case, one would expect the product distributions in Figure 5c,d to mirror those in Figure 5a,b. Although such activation may certainly contribute to 4-aminophthalimide 3-position thionation and reactivity in general, it is perhaps not significant enough to overcome the regiochemical implications associated with LR.

Additionally, a prominent solvent effect disparity between 1,4-dioxane and toluene influencing 3-thionation is unlikely, as one would presume a more marked discrepancy in the corresponding traces shown in Figure 5b,d. However, the modest selectivity differences seen in pomalidomide thionation (Table 1) may help to illuminate the importance of aniline hydrogen bonding on the associated regiochemical bias, as the notably stronger hydrogen-bond-accepting pyridine deteriorates the reaction rate and selectivity relative to weaker acceptors like 1,4-dioxane.[18] While a full and thorough mechanistic investigation is beyond the scope of this preliminary (and largely empirical) study, the selectivity afforded here is quite striking and likely due to a complex amalgamation of steric and electronic properties coupled with the peculiarities of the specific thionating species involved. Further mechanistic analyses and new thionated materials are currently being explored.

Conflict of Interest

The authors declare no conflict of interest

Funding Information

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Acknowledgment

N.G. has a patent on compounds **3** and **4**, which is assigned to the National Institute on Aging, NIH: *Thalidomide Analogs and Methods of Use. US 10,730,835 B2*, **2020**. The authors wish to thank Dr. Shelley Jackson from the NIH NIDA Structural Biology Core for assistance with the high-resolution mass spectrometry analyses.

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1720460.

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(4) See the Supporting Information for LC/MS profiles of a typical reaction with Lawesson's Reagent compared with our new method.

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(10) **Thionation of Pomalidomide: General Procedure**
Representative conditions: A vial was charged with 25 mg of pomalidomide (1 equiv) and the appropriate thionating reagent (1.5 equiv of P_4S_{10}-pyridine, or 0.75 equiv of P_4S_{10}). The vial was sealed and placed under N_2. The indicated solvent (2.5 mL) was injected and the mixture was stirred vigorously while the contents were briefly degassed. Then, the reaction was heated to 100 °C and monitored by LC/MS. See the Supporting Information for complete details.

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(12) We did not isolate these materials or characterize their structures. However, given the nature of the reaction, the observed masses seemed plausible for the incorporation of the indicated amount of sulfur. See the Supporting Information for examples.

(13) Fortunately, even after 8 hours of reaction time, the P_4S_{10}-pyridine complex/1,4-dioxane system demonstrated 3,6′-DT-pom **3** as the overwhelmingly favored dithionated product and with only traces of other thionated isomers. As was expected, reactions conducted solely with P_4S_{10} were driven significantly towards presumed trithionated materials in the same period of time. See the Supporting Information for examples.

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(16) **4-Amino-2-(2-oxo-6-thioxopiperidin-3-yl)-3-thioxoisoindolin-1-one (3): Typical Procedure**
A vial was charged with pomalidomide (**1**; 25 mg, 0.091 mmol, 1.0 equiv), P_4S_{10}–pyr (52 mg, 0.14 mmol, 1.5 equiv), then sealed and placed under N_2. 1,4-Dioxane (2.5 mL) was added by injection and the mixture was briefly degassed, then stirred and heated at 100 °C. After 8 hours, the reaction changed from cloudy yellow through clear orange to deep red. The mixture was cooled to r.t., and the red reaction liquor was decanted, evaporated to dryness, and dissolved in EtOAc. Then, the dark oily residue remaining in the original reaction vial was stirred gently with EtOAc and sat. aq $NaHCO_3$. The resulting two-phase mixture was transferred to a separatory funnel and combined with the previous EtOAc solution. The aqueous phase was carefully drained off, and the organic phase was washed successively with water, 0.5 M HCl, and brine, then dried (Na_2SO_4) and concentrated. The crude material was purified by HPLC to give a bright-orange powder; yield: 8.0 mg (29%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.63 (s, 1 H), 7.65 (br s, 2 H), 7.50–7.40 (m, 1 H), 7.18 (d, *J* = 8.5 Hz, 1 H), 7.03 (br s, 1 H), 5.64–5.40 (br m, 1 H*), 3.26–2.78 (br m, 2.5 H*), 2.02 (m, 1 H) (* difficult to resolve). HRMS (MALDI): *m/z* [M + Na]$^+$ calcd for $C_{13}H_{11}N_3NaO_2S_2$: 328.01849; found: 328.01880. Structural assignments are further supported and confirmed by single-crystal X-ray analysis (see ref. 19 and Supporting Information).

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(19) CCDC 2074092 (**3**), 2074088 (**4**), 2074087 (**14**), 2074089 (**16**), 2074090 (**18**), and 2074091 (**20**) contain the supplementary crystallographic data for the compounds listed. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.