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Synthesis of Protected 3,4- and 2,3-Dimercaptophenylalanines as Building Blocks for *Fmoc*-Peptide Synthesis and Incorporation of the 3,4-Analogue in a Decapeptide Using Solid-Phase Synthesis

Isita Banerjee, Keshab Ch Ghosh, Emmanuel Oheix, Marion Jean, Jean-Valère Naubron, Marius Réglier, Olga Iranzo,* and Surajit Sinha*

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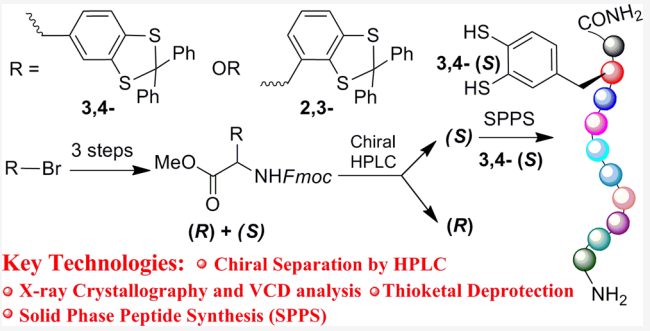
ABSTRACT: 3,4-Dimercaptophenylalanines and 2,3-dimercaptophenylalanines have been synthesized for the first time by nucleophilic substitution of a protected aminomalonate on 3,4- and 2,3-dimercaptobenzyl bromide derivatives. The dithiol functions were protected as thioketals, and the key precursors, diphenylthioketal-protected dimercaptobenzyl bromides, were synthesized via two distinct routes from either dihydroxy benzoates or toluene-3,4-dithiol. Racemic mixtures of the fully protected amino acids were separated by chiral HPLC into the corresponding enantiomers. The absolute configuration of both 3,4- and 2,3-analogues could be assigned based on X-ray crystallography and VCD/DFT measurements. Thioketal groups were deprotected upon reaction with mercury oxide and aqueous tetrafluoroboric acid followed by treatment with H_2S gas under an argon atmosphere to obtain the corresponding dimercapto amino acids. The optically pure L-*Fmoc*-protected 3,4-analogue (*S*- enantiomer) was successfully incorporated into a decapeptide using standard solid-phase peptide synthesis. Therefore, dithiolene-functionalized peptides are now accessible from a simple synthetic procedure, and this should afford new molecular tools for research into the catalysis, diagnostic, and nanotechnology fields.



INTRODUCTION

Cysteine is the only canonical amino acid with a thiol function, and this allows this residue to play a unique role in protein functions (regulatory, catalytic, or metal ion binding).[1] The ability of the thiol group to form reversible disulfide bridges confers to cysteine a key role in the conformational structure of peptides or proteins.[2] Over the past several decades, cysteine is also very often used in the thioester method for the chemical synthesis of peptides and proteins through native chemical ligation (NCL) as well as for their selective modification.[3] On the other hand, the nucleophilicity of its conjugated base (cysteinate) endows proteins containing cysteines with special functional properties. Among them, the reactivity as a transient nucleophile in numerous enzymatic processes or as a soft ligand having a high affinity for metals (Fe, Cu, Hg, Cd, Zn, Mo, W, etc.) is certainly the most significant.[1,4] Additionally, cysteine or cysteine-containing peptides are useful in the generation of functionalized nanoparticles.[4b,d,e]

Considering the importance of cysteine and the scarcity of thiol-containing amino acids, the synthesis of new mercapto amino acid derivatives is highly desirable. Under these grounds, a number of noncanonical thiol-containing amino acids have been reported[5] in which cysteine surrogates have been incorporated through the modification of naturally occurring amino acids with the main goal of overcoming the limitation of *N*-terminal cysteine-mediated NCL for synthesizing large peptides/proteins. Surprisingly, there are very few reports in the case of dithiol-containing amino acids.[6] Heinis et al. have prepared two L-4(*R*/*S*),5-dithionorvaline diastereoisomers as a surrogate for the cysteine-cysteine fragment.[6a] This modification allows directing dithiol bond formation leading to specific peptide tertiary structures. Ghirlanda et al. have reported the preparation of an L-5,6-dithioleucine amino acid, which was incorporated in a helical peptide to anchor a diiron hexacarbonyl cluster to a model helical peptide and mimic the hydrogenase active site for hydrogen production.[6b] Wang et al. have synthesized dithiol-functionalized L-tyrosine through the incorporation of 2-methylpropane-1,3-dithiol linker via the etherification of tyrosine OH.[6c]

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Indeed, to the best of our knowledge, no amino acid bearing the dithiolene (alkene-1,2-dithiolate) function has been reported to date. These new building blocks can facilitate the introduction of dithiol groups and endow peptides/proteins with new structures and/or functionalities, as previously mentioned. Sinha's group has focused on the synthesis of thiol and selenol-containing noncanonical amino acids for the past few years.[7] In this context, we describe here the synthesis of suitably *Fmoc*-protected 3,4-dimercaptophenylalanine ($\mathbf{F^{3,4\text{-}dt}}$) and 2,3-dimercaptophenylalanine ($\mathbf{F^{2,3\text{-}dt}}$) as building blocks to introduce the dithiolene group in peptides (Figure 1). The solid-phase synthesis of a decapeptide containing the dithiolene functionalities is reported.

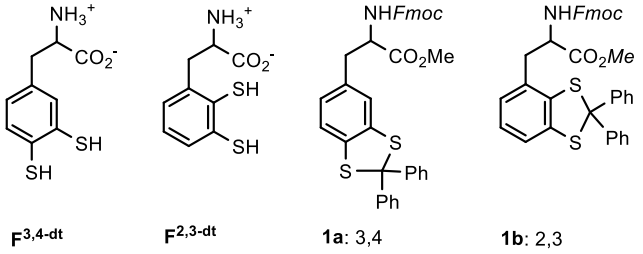


Figure 1. Structures of 3,4- and 2,3-dimercaptophenylalanine residues ($\mathbf{F^{3,4\text{-}dt}}$ and $\mathbf{F^{2,3\text{-}dt}}$) and the fully protected amino acid building blocks (**1a** and **1b**).

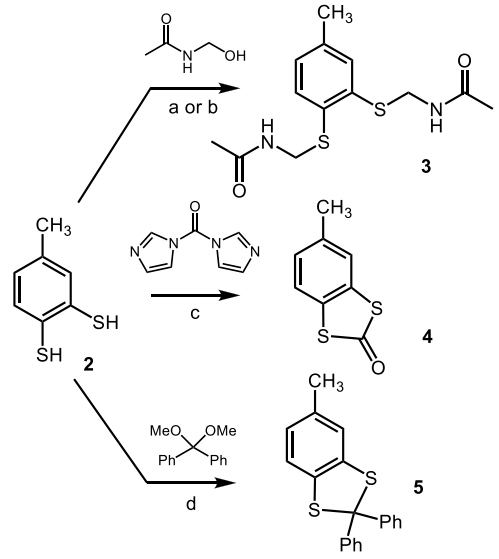
RESULTS AND DISCUSSION

Pathways in the Protection of Dithiolene Function. Dithiolenes are reactive functions (e.g., nucleophile, reductant, and metal binding), and finding a protecting group for the dithiolene function compatible with standard peptide synthesis protocols constituted our first priority. Acetamidomethyl (Acm) groups are commonly used in peptide synthesis to protect thiols[8] or dithiols,[6a] and they constitute promising candidates. Moreover, the dithiol function can be regenerated upon treatment with an excess of iodine. Using commercially available toluene-3,4-dithiol (**2**), we attempted the protection of dithiolene with Acm groups (Scheme 1, pathways a and b). Following a reported procedure,[9] the deprotected compound (**3**) was obtained in a moderate 45% yield. When the reaction was carried out in the presence of TMSCl,[10] **3** was isolated in 48% yield. Discouraged by these initial results, we sought alternatives.

Dithiolenes functions can also be protected via *S*-acylation or the formation of dithiocarbonates. In the first case, the conventional deprotection involves the use of a highly basic and poorly selective alkoxide. In contrast, dithiocarbonate can be readily converted to the related dithiols by hydrolysis. Therefore, using the second alternative, compound **4** was obtained in 92% yield upon reacting **2** with 1,1′-carbonyldiimidazole (Scheme 1, pathway c).

We suspected that the dithiocarbonate function might be labile in basic conditions; therefore, compound **4** was treated with 20% piperidine in DMF, a mixture conventionally used to deprotect *Fmoc*-protected amines in *Fmoc*-peptide chemistry. After a 1 h reaction and drying *in vacuo*, ^{1}H NMR analysis of the crude mixture indicated the presence of several products. Therefore, dithiocarbonates have poor compatibility with *Fmoc* chemistry, and we sought an alternative protection strategy.

Scheme 1. Protection of Aryldithiol[a]



[a]Reagents and conditions: (pathway a) *N*-(hydroxymethyl)acetamide (3.2 equiv), conc. HCl, MeOH, RT, 20 h, 45%; (pathway b) *N*-(hydroxymethyl)acetamide (3.2 equiv), TMSCl (2 equiv), THF, RT, 16 h, 48%; (pathway c) carbonyldiimidazole (1.2 equiv), THF, RT, 18 h, 92%; (pathway d) $Ph_2(OMe)_2$ (1.2 equiv), p-TSA (10 mol %), toluene, 12 h, reflux, 98%.

Dithiol compounds are used to protect aldehyde or ketones as thioketals.[11] Looking from the opposite perspective, it might be possible to protect dithiolene as thioketal upon reaction with a suitable aldehyde or ketone, provided suitable deprotection conditions can be found. Surprisingly, there is no reported method for aryldithiols protection as a thioacetal or thioketal. Since we were interested in limiting the number of chiral centers in the target amino acid, we decided to use a symmetrical ketone. Following a reported thioketalization method,[12] we tried to prepare compound **5** by reacting **2** with benzophenone in the presence of a catalytic amount of iodine, but the expected compound **5** was obtained in poor yield (38%). A slightly higher yield (45%) was obtained for the acid-catalyzed (*p*-toluenesulfonic acid) ketalization in dry toluene after 15 h reflux. Different conditions ($BF_3{\cdot}OEt_2$, 53% and $FeCl_3$, 57%) (Table S1) were exploited, but the yield has not been improved. In contrast, replacing benzophenone with its dimethyl ketal derivative[13] afforded compound **5** in 98% yield (Scheme 1, pathway d). Following this procedure, dithiolenes can be conveniently protected as thioketals, and, importantly, dithiolene functions can be regenerated selectively.

Synthesis of Racemic Mixtures 1a and 1b. The specific aim of this work is to prepare $\mathbf{F^{3,4\text{-}dt}}$ and $\mathbf{F^{2,3\text{-}dt}}$ as suitable building blocks for *Fmoc*-peptide synthesis. Toward this goal, the target molecules, **1a** and **1b**, will include methyl ester-protected carboxylates, *Fmoc*-protected amines, and dithiol side chains protected as thioketals. Our initial approaches toward the asymmetric synthesis of $\mathbf{F^{3,4\text{-}dt}}$ from L-DOPA (Scheme S1) and $\mathbf{F^{2,3\text{-}dt}}$ based on the Negishi coupling between dimercapto-iodobenzene and (*R*)-methyl 2-((*tert*-butyloxycarbonyl)-amino)-3-iodopropanoate (Scheme S2) were not successful and encountered a number of problems, while handling the reactions in the presence of dithiol. Therefore, we focused on obtaining the racemic mixtures. Our retrosynthetic pathway toward racemic compounds **1a** and **1b** is in Scheme 2. The

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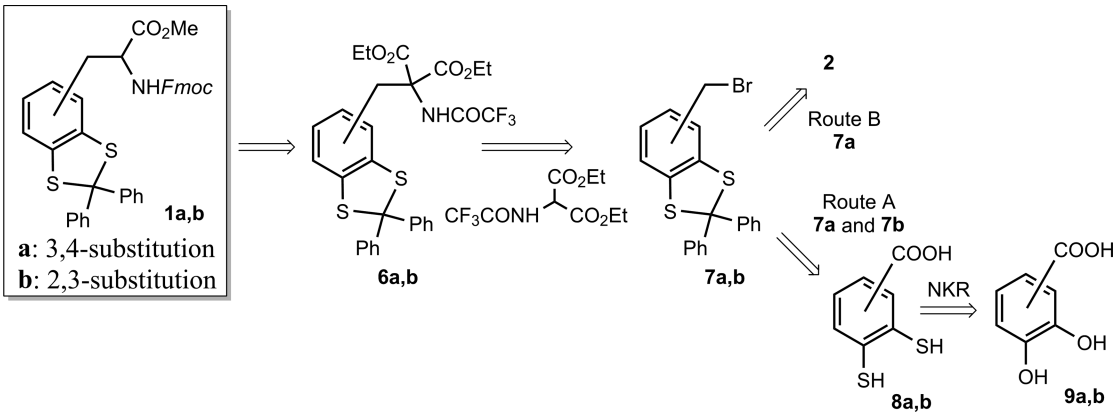
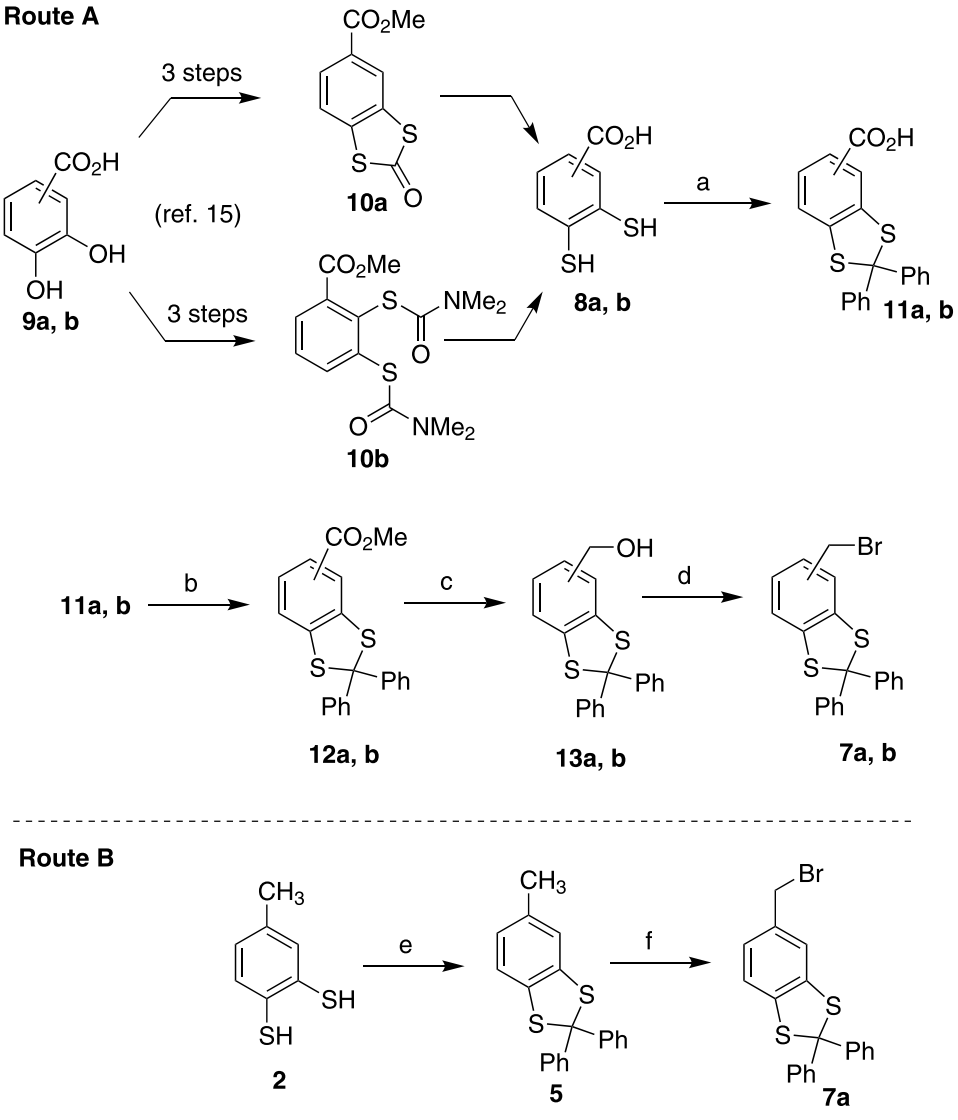
Scheme 2. Retrosynthetic Route toward Racemic Compounds 1a and 1b



Scheme 3. Preparation of Compounds 7a and 7b[a]



[a]Reagents and conditions: (a) $Ph_2(OMe)_2$ (1.2 equiv), *p*-TSA (10 mol %), toluene, reflux, 12 h, **11a** = 84%, **11b** = 81% (from **10a**/**10b**); (b) K_2CO_3 (2 equiv), CH_3I (4 equiv), DMF, 0°C to RT, 12 h, **12a** = 84%, **12b** = 81%; (c) $AlCl_3$ (1.5 equiv), $LiAlH_4$ (3.8 equiv), Et_2O, 0 °C, 5 min, **13a** = 92%, **13b** = 91%; (d) PBr_3 (1 equiv), Et_2O, 0 °C to RT, 4 h, **7a** = 85%, **7b** = 85%; (e) $Ph_2(OMe)_2$ (1.2 equiv), *p*-TSA (10 mol %), toluene, 12 h, reflux, **5** = 98%; (f) NBS (1.1 equiv), CH_2Cl_2, 1.5 h, reflux, **7a** = 65%.

coupling of *N*-protected aminodiethylmalonate with suitably dithiol-protected dimercaptobenzyl bromide shall afford **6a** and **6b**, which, upon hydrolysis and reprotection, lead to **1a** and **1b**. The key precursors dimercaptobenzyl bromides (**7a**/**7b**) could

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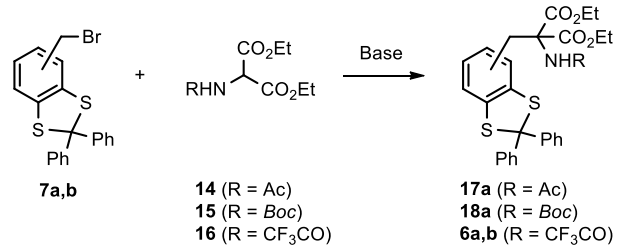
be synthesized from dimercapto benzoates (**8a**/**8b**), which, in turn, can be prepared from the corresponding dihydroxy benzoates (**9a**/**9b**) by Newman−Kwart rearrangement (NKR)[14] (Scheme 2, route A). In this reaction, intramolecular migration of *O*-thiocarbamates at high temperatures leads to *S*-thiocarbamates.[14] Alternatively, 3,4-dimercaptobenzyl bromide (**7a**) can be prepared directly from toluene-3,4-dithiol **2** (Scheme 2, route B).

Starting from affordable substrates **9a** and **9b**, compounds **7a** and **7b** were obtained in 8 steps in 21% (**7a**) and 18% (**7b**) overall yields, respectively (Scheme 3, route A). Dimercaptobenzoic acids **8a** and **8b** were synthesized from the corresponding 3,4- and 2,3-dihydroxybenzoic acids (**9a**,**b**), respectively, via the formation of NKR products, following the previously reported procedures (Scheme S3).[15] Compounds **10a** and **10b** were hydrolyzed by NaOH solution to obtain dimercaptobenzoic acids **8a**[15b] and **8b**,[15] which were then directly used for dithiolene protection as thioketals, to afford **11a** and **11b**, respectively (Scheme 3).

The dimercaptobenzyl bromides **7a** and **7b** were obtained in three steps via the formation of the esters (**12a**/**12b**) and alcohol derivatives (**13a**/**13b**) (Scheme 3). The methyl benzoates **12a** and **12b** were prepared using the standard method. The optimal ester reduction procedure involved in situ preparation of AlH_3 and afforded the desired alcohols (**13a**/**13b**) at 0 °C within 10 min in quantitative yields. The pure reduction products were not obtained when $LiAlH_4$ or $LiBH_4$ was used. Finally, PBr_3-mediated bromination of **13a** and **13b** was achieved in anhydrous diethyl ether at 0 °C for 4 h, affording **7a** and **7b** in 85% yields (Scheme 3, route A). The solvent has an important role in this reaction: when the reaction was performed in dichloromethane, **7a** was obtained, whereas **7b** was decomposed in the reaction mixture. CBr_4/PPh_3 led to the formation of a number of unidentified products. As an alternative, compound **7a** could be obtained in two steps and 64% overall yield starting from the commercially available compound **2** (Scheme 3, route B). The first step involved the previously described protection of **2** as **5**, and the second step involved the bromination with *N*-bromosuccinimide.[16] The bromination of Acm-protected **3** was also attempted under similar conditions; unfortunately, we ended up with a large number of unidentified products. During the preparation, crystals of **7a** and **7b** suitable for X-ray diffraction were obtained, and the structure of these compounds are reported (Figure S1, Tables S2 and S3).

For the next step, different conditions were screened in order to optimize the coupling of dimercaptobenzyl bromides with malonate esters in the presence of a base (Table 1). Different malonates were tested, including acetamido (**14**), *Boc*-protected (**15**), and trifluoroacetamido malonates (**16**). Compounds **15** and **16** were synthesized from diethyl(amino)malonate hydrochloride, which was prepared using the literature procedure.[17] The reaction between **7a** and **14** in the presence of NaH run at room temperature (RT) for 12 h in dry DMF led to the desired product **17a** in 65% yield (Table 1, entry 1). However, we were unable to obtain the amine-deprotected product through the hydrolysis of the acetamide bond in the presence of NaOH in water/THF mixture. Hence, we used malonates **15** and **16**, where the Boc group can be removed under 20% TFA in DCM and trifluoroacetamide can be removed in the presence of LiOH in a water/THF mixture. The reaction of **7a** with diethyl(*Boc*-amino)malonate **15** was first attempted at RT for 12 h in the presence of NaH and led to the formation of several unidentified

Table 1. Standardization of Reaction Conditions[a]



7a,**b**

14 (R = Ac)
15 (R = *Boc*)
16 (R = CF_3CO)

17a (R = Ac)
18a (R = *Boc*)
6a,**b** (R = CF_3CO)

entry	SM	Re	base	S	*T* (°C)	*t* (h)	*Y* (%)
1	**7a**	**14**	NaH	DMF	RT	12	65
2	**7a**	**15**	NaH	DMF	RT	12	32
3	**7a**	**15**	NaH	DMF	70*	12	*b*
4	**7a**	**15**	NaOEt	EtOH	0	3	*b*
5	**7a**	**15**	Cs_2CO_3	DMF	70*	12	*b*
6	**7a**	**16**	NaH	DMF	RT	12	40
7	**7a**	**16**	NaH	DMF	RT	6	82
8	**7b**	**16**	NaH	DMF	RT	6	75

[a]Reaction conditions: substrate (1.0 mmol), malonate (1.0 mmol), base (1.2 mmol), dry solvent (5 mL) were added at 0 °C (except for *) under an inert atmosphere, allowing the mixture to warm to the reaction temperature; SM= starting material; Re = reagent; S = solvent; *T* = reaction temperature; *t* = reaction time; *Y* = yields of isolated pure products in %. [b]The reaction led to several unidentified products and/or starting material.

products and unreacted starting material (Table 1, entry 2). No quantitative yield of **18a** could be attained upon increasing the temperature or replacing NaH for NaOEt or Cs_2CO_3 (Table 1, entries 3−5).[18] In contrast, reacting **7a** with **16** at RT in dry DMF containing NaH led to the formation of compound **6a** in 40% yield (Table 1, entry 6). The optimal reaction time was found to be 6 h and led to **6a** in 82% yield (Table 1, entry 7). Following these conditions, the 2,3-substrate **6b** was synthesized in 75% yield (Table 1, entry 8).

Then, the hydrolysis of ester function, decarboxylation reaction, and hydrolysis of the trifluoroacetamide group were performed in one pot following our previously reported procedure.[19] Accordingly, compounds **6a** and **6b** were treated with aqueous LiOH solution in THF (1:1) at 70 °C for 12 h. The crude products were directly reacted with *Fmoc*-OSu ester in standard conditions to isolate the *Fmoc*-protected amino acids **19a** and **19b** in 75 and 72% yields, respectively. Treatment with MeI at RT for 12 h in the presence of K_2CO_3 afforded the methyl esters **1a** and **1b** in 78 and 77% yields, respectively (Scheme 4). The free acids **19a** and **19b** can be recovered upon treatment with $CaCl_2$ and LiOH in a mixture of water, isopropanol, and tetrahydrofuran.[20]

Chiral Separation and Assignment of Absolute Configuration. The racemic mixtures contained ∼10% side products, which were either removed before (**1a**) or during the chiral separation (**1b**). The racemic mixtures were then eluted isocratically with heptane/isopropanol/dichloromethane mixtures on an analytical Chiralpak IF column. Both chromatograms display two peaks with similar integral values (Figure 2 and Figure S2 for **1b**). Using these conditions, the fractions were separated on a preparative Chiralpak IF column, and individual enantiomers (enantiomeric excess > 99%) were characterized by electronic circular dichroism (Figure S3 for **1a**, Figure S4 for **1b**) and optical rotation measurements (Table S4 for **1a**, Table S5 for **1b**).

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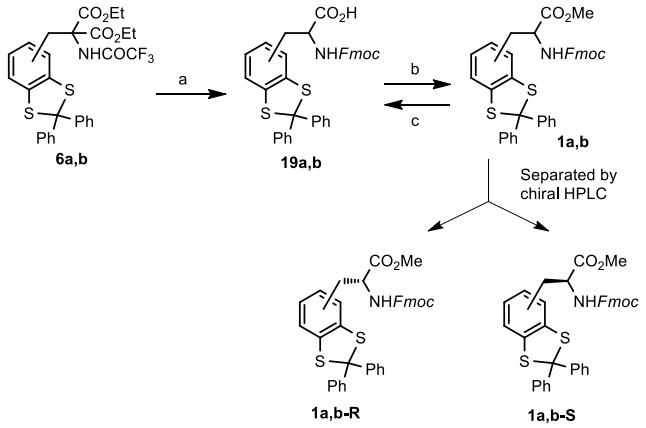
Scheme 4. Synthesis of Protected Dimercaptophenylalanines 1a and 1b[a]



[a]Reagents and conditions: (a) (i) $LiOH{\cdot}H_2O$ (12 equiv), H_2O−THF (1:1), 70 °C, 12 h; (ii) $NaHCO_3$, *Fmoc*-OSu (2 equiv), dioxane−H_2O, RT, 12 h, **19a** = 75%, **19b** = 72%; (b) CH_3I (4 equiv), K_2CO_3 (2 equiv), DMF, RT, 12 h, **1a** = 78%, **1b** = 77%; (c) $CaCl_2$ (16 equiv), $LiOH{\cdot}H_2O$ (4 equiv), isopropanol/THF/H_2O (3:1:1.5), 2.5 h, **19a** = 98%, **19b** = 90%.

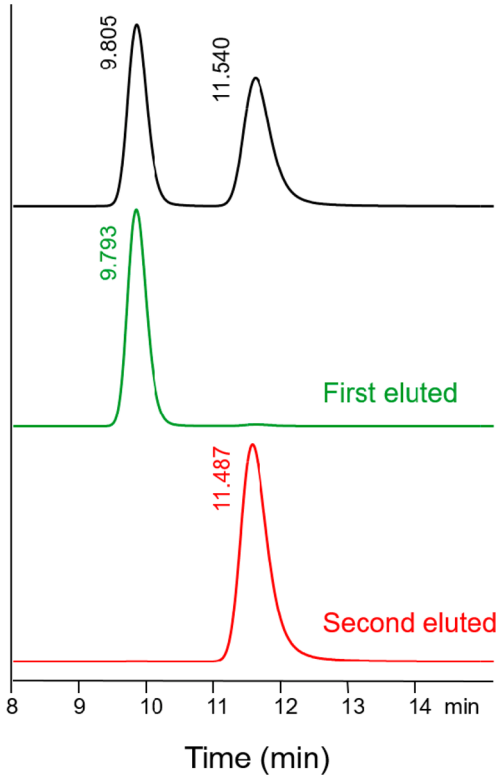


Figure 2. HPLC chromatogram (detection at 254 nm, 1 mL/min) recorded upon isocratic elution with heptane/isopropanol/dichloromethane (80:10:10) on a analytical Chiralpak IF column of a (top) racemic mixture of compound **1a**, (middle) purified enantiomer **1a**-*R* (bottom) and purified enantiomer **1a**-*S*.

In the case of **1a**, thin needles of the enantiomer eluting first in HPLC were obtained, and X-ray diffraction measurements allowed the determination of the structure and the absolute configuration as (*R*) (Figure 3, Figures S5 and S6, Table S6). Hence, **1a**-*R* defines the compound eluting at 9.8 min in chiral HPLC (Figure 2). The absolute configuration assignment

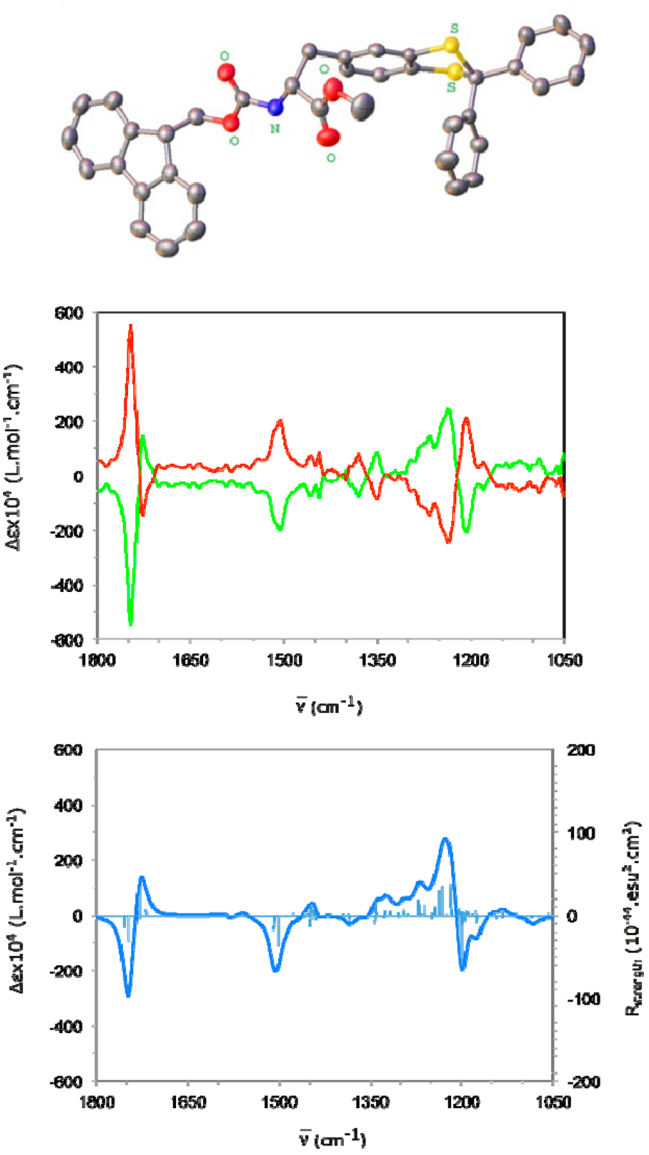


Figure 3. (Top) ORTEP representation of the X-ray structure of the first eluted enantiomer of compound **1a** with 50% probability thermal ellipsoids and partial atom labeling scheme (hydrogen atoms are omitted for clarity). (Middle) Experimental VCD spectra recorded in CD_2Cl_2 at 298 K of (first eluted)-**1a** (green) and (second eluted)-**1a** (red). (Bottom) Calculated averaged VCD spectrum for **1a**-*R* (dark blue).

established by X-ray diffraction was consolidated by a vibrational circular dichroism (VCD) analysis. The VCD spectrum calculated (DFT) for the (*R*) enantiomer is in very good agreement with the measured spectrum of the first eluted enantiomer. The VCD spectrum of the first and second eluted enantiomers have been recorded in CD_2Cl_2 and compared to the calculated averaged spectrum of the **1a**-*R* enantiomer (Figure 3). Calculations were performed using the density functional theory (DFT) with the B3LYP functional and with the 6-311G(d,p) basis set. The polarized continuum model SMD has been used for the solvent effects. The conformational flexibility of **1a** has been studied: 9 conformations with the Boltzmann population higher than 3% have been retained to build the averaged VCD spectrum (Figures S7−S9, Table S7).

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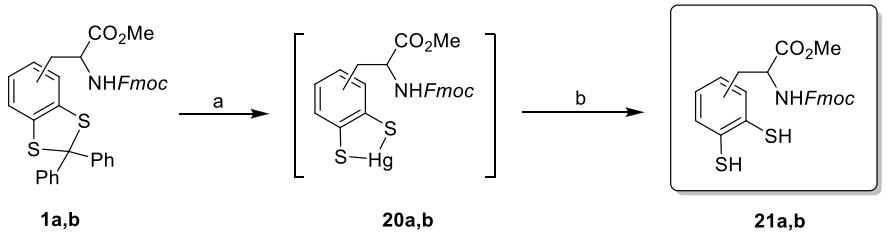
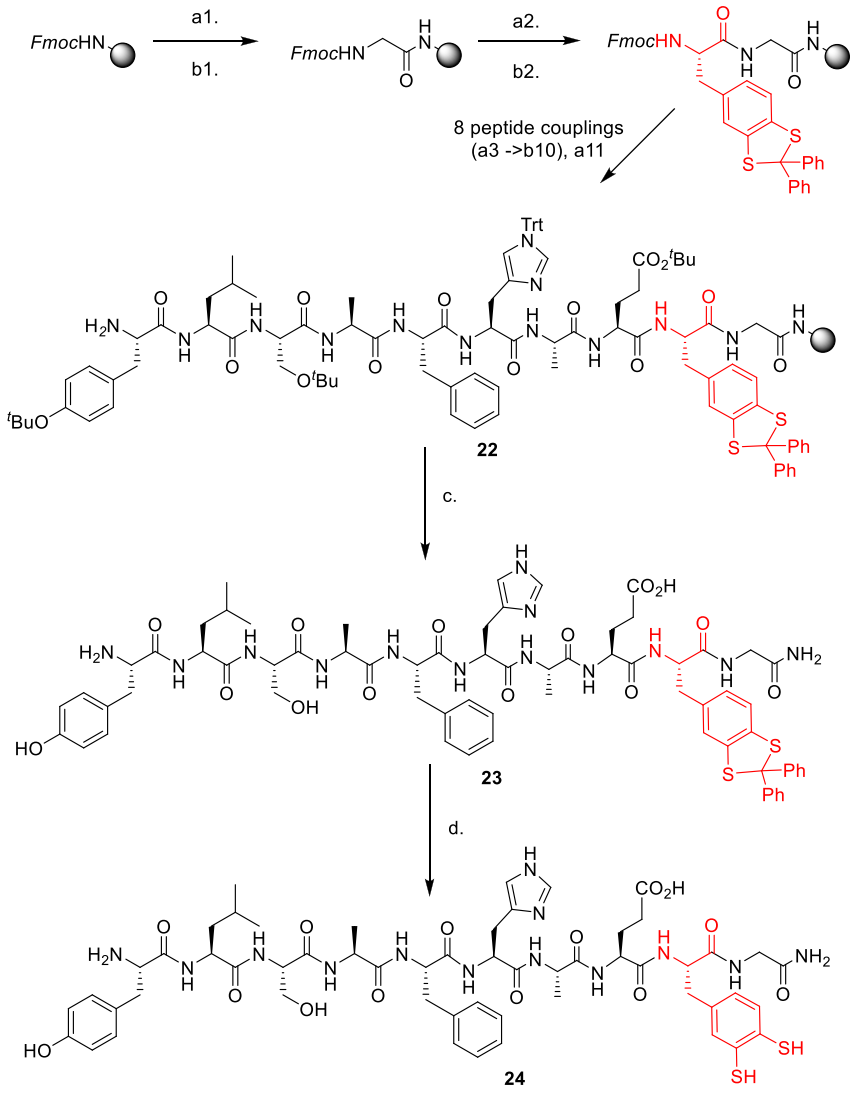
Scheme 5. Synthesis of Dimercaptophenylalanines[a]



1a,b 20a,b 21a,b

[a]Reagents and conditions: (a) HgO (1.5 equiv), HBF_4 35 wt % in H_2O (6.8 equiv), THF, RT; (b) H_2S (g), EtOAc, RT, 4 h, Ar maintained by Schlenck line, **21a** = 44%, **21b** = 43%.

Scheme 6. Synthesis of a Decapeptide Containing the Dithiol Function[a]



[a]Reagents and conditions: (a) 20% piperidine in DMF (2 × 4.5 mL), 3 and 10 min; (b) *Fmoc*-protected amino acids (5 equiv) DIEA (10 equiv), HBTU (4.9 equiv), DMF, 75°C, 5 min; exceptions: (b2) (i) **19a**-*S* (2 equiv), HBTU (2 equiv), DIEA (4 equiv), DMF, RT, 60 min; (ii) **19a**-*S* (1.1 equiv), HBTU (1.1 equiv), DIEA (2.2 equiv), DMF, RT, 1 h; (b5) *Fmoc*-His(Trt)−OH (5 equiv) DIEA (10 equiv), HBTU (4.9 equiv), DMF, 50 °C, 6.5 min; (c) TFA/TIS/water (95/2.5/2.5), RT, 2 h; (d) (i) HgO (1.5 equiv), HBF_4 35 wt % in H_2O (6.8 equiv), RT, THF, 2 h; (ii) H_2S gas, EtOAc/MeOH (95:5), RT, 4 h.

As discussed earlier for **1a**, the racemic mixture of **1b** was separated into the corresponding enantiomers (**1b**-*R* and **1b**-*S*) by chiral HPLC on a Chiralpak ID (Figure S2). Fractions of two enantiomers eluting at 5.51 and 6.48 min were collected and characterized by optical rotations (Table S5) and circular dichroism (Figure S4). As for **1a**, the VCD was used to establish the absolute configuration (*R*) of the first eluted enantiomer of **1b** unambiguously (Figures S10 and S11, Table S8).

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In order to use these building blocks in peptide synthesis, the acid function must be deprotected. For this, compounds **1a**-*R* and **1a**-*S* were individually treated with $CaCl_2$ and LiOH solution[20] to afford **19a**-*R* and **19a**-*S* in 98% and 90% yields, respectively, following the protocol of racemic mixtures reported in Scheme 4. Analytical chiral HPLC confirmed that the deprotection conditions did not lead to racemization as the enantiomeric excess of both **19a**-*R* and **19a**-*S* was >99% (Figure S12).

Deprotection of the Dithiolene Function via Thioketal Hydrolysis. The dithiol side-chain deprotection is best achieved via thioketals hydrolysis. In order to drive the reaction to completion, common methods involve the consumption of the dithiols side product as it forms (via alkylation, oxidation, or metal complexation).[21] In our case, the dithiol is the desired product; hence, a two-step process was used: (i) treatment with Hg^{II} and acid leading to the formation of the Hg^{II} complex; (ii) removal of the Hg^{II} ion using an excess of H_2S. Following a reported procedure,[22] deprotection of racemic amino acids **1a** and **1b** was attempted with $HgCl_2$ in acetonitrile−water or in pure trifluoroacetic acid, but no deprotection was observed as TLC monitoring indicated that no benzophenone side product was formed. The replacement of mercury for less toxic reagents such as $AgNO_3$,[22] $AgNO_2$,[22] or Na/naphthalene[23] did not give any desired product. Treatment with triethylsilane in 50% TFA[24] gave a trace amount of product. Treatment with HgO afforded partial conversion in acetonitrile−water or in trifluoroacetic acid/THF. Complete deprotection was obtained (monitored by TLC) using HgO and 35% aqueous HBF_4 in THF, leading to the mercury complex **20** of the corresponding amino acids **1a** and **1b** (Scheme 5). Successive trituration of the crude mixture with water followed by petroleum-ether to remove HBF_4 and benzophenone, respectively, gave rise to the Hg complex **20** as a pale yellow precipitate. Next, H_2S gas was flown into a suspension of **20** in EtOAc under an argon atmosphere (maintained by Schlenk line) and successfully isolated the desired products **21a** and **21b** after centrifugation. We could not isolate the product when the reaction was carried out under an Ar balloon. It is worth mentioning here that the isolation of dimercaptopropanol from Hg complex by the decomposition of $H_2S(g)$ was not possible earlier; perhaps, the presence of oxygen may have a substantial effect on the isolation of free dithiol.[25] As X-ray photoelectron spectroscopy (XPS) is a useful technique for the detection of inorganic elements, we performed XPS analysis of the amino acids **21a** and **21b**. The core-level spectra showed two peaks with the highest relative intensities at binding energies 163.5 and 164.6 eV, which correspond to S $2p_{3/2}$ and S $2p_{1/2}$ core lines, respectively (Figures S13 and S14). These values were in good agreement with the reported data of sulfur.[26] However, no band was observed for the Hg 4d core level in the spectral window 383−353 eV, consistent with the absence of Hg-containing impurities.[26,27] Since the absorption length of core-level electrons depends on the kinetic energy, the Hg 4d level is preferred over the Hg 4f level for component analysis. The presence of free −SH was confirmed by IR spectroscopy, where peaks at 2555 cm^{-1} for 3,4-amino acid (**21a**) and 2549 cm^{-1} for 2,3-amino acid (**21b**) were obtained and further characterized by NMR and HRMS. In the case of 3,4-dimercaptophenylalanine derivative **21a**, sometimes, we observed a mass of 949.1724 $[M + Na]^+$, corresponding to dimerization product as a single peak in HRMS analysis (Supporting Information).

Solid-Phase Peptide Synthesis of a Decapeptide Containing L-Phe$^{3,4\text{-dt}}$ (= L-F$^{3,4\text{-dt}}$). In order to test the applicability of the noncanonical amino acid L-**Phe**$^{3,4\text{-dt}}$ in solid-phase peptide synthesis, we prepared the decapeptide H_2N-Tyr-Leu-Ser-Ala-Phe-His-Ala-Glu-Phe$^{3,4\text{-dt}}$-Gly-$CONH_2$ bearing only L-residues and different side-chain functionalities. At first, sequence H_2N-Tyr(O^tBu)-Leu-Ser(O^tBu)-Ala-Phe-His(Trt)-Ala-Glu(O^tBu)-Phe$^{3,4\text{-dt}}$(CPh_2)-Gly-resin was assembled on rink amide resin by mixed manual and automated solid-phase peptide synthesis using standard protocols (see Experimental Section). In this case, the noncanonical amino acid **19a**-*S* was incorporated by the manual synthesis in the second position starting from C-terminus in order to test its stability during 8 successive amino acid couplings (Scheme 6). After final *Fmoc* deprotection, decapeptide **22** was simultaneously deprotected (except for the thioketal group) and cleaved from the resin to obtain **23**. Using the method previously described for **21a** and **21b**, the thioketal function was hydrolyzed to obtain the deprotected decapeptide **24** (Scheme 6). In order to eliminate any Hg impurities, decapeptide **24** was then treated with dithiothreitol (DTT, 5 equiv) and passed through a dialysis bag for 48 h. The absence of Hg was confirmed by XPS analysis, displaying no peak for the Hg 4d core level in the range 383−350 eV (Figure S15). Peptide (**24**) was characterized by MALDI-TOF, and its purity (>95%) was determined by HPLC using a SPOLAR C18 S5 column (Figure S16). In IR spectroscopy, a peak at 2535 cm^{-1} was observed, which strongly suggested the presence of the free −SH group. In any case, the preparation of peptide **23** demonstrated that the protection scheme described herein is compatible with standard solid-phase peptide synthesis, and the dithioketal deprotection could be selectively achieved in solution affording **24** as a free dithiolene-containing peptide. Before the treatment with DTT, the crude decapeptide was subjected to MALDI TOF mass analysis, and four peaks were found at m/z values of 1227.613 (calcd 1226.4739) and 1244.351 (calcd 1242.4478) corresponding to $[M + Na]^+$ and $[M + K]^+$, respectively. The other two masses present at m/z values of 1259.337 (calcd 1258.4460) and 1275.481 (calcd 1274.4199) corresponded to persulfides $[M - H + SH + Na]^+$ and $[M - H + SH + K]^+$, respectively, as expected based on the literature reports.[28] DTT-mediated persulfide reduction gave the desired product **24**.

CONCLUSION

We describe herein a convenient method for the preparation of racemic 3,4- and 2,3-dimercaptophenylalanines, as new entries in the noncanonical amino acid library. The key synthetic step involves the deprotection of the thioketal function to obtain free dimercaptophenylalanines. A method for the chiral resolution using preparative chiral HPLC is also reported, and the absolute configuration of the enantiomers could be assigned based on X-ray structure and vibrational circular dichroism spectra correlated to DFT calculations. These building blocks are compatible with *Fmoc*-peptide synthesis, and a decapeptide including the L-**F**$^{3,4\text{-dt}}$ residue was prepared, thus confirming the viability of our dithiolene protection scheme. Indeed, the thioketal deprotection was carried out successfully to obtain the dithiolene-containing decapeptide. After purification, XPS analysis indicated that the dithiolene-containing amino acids and decapeptide were free from Hg contamination. We are currently developing new peptide sequences whose applications will be exploiting the chemical and physical properties of the dithiolene side chains.

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EXPERIMENTAL SECTION

General Methods. All reagents were purchased from commercial sources and used without further purification, unless otherwise stated. Petroleum ether (Pet-ether) refers to the fraction of petroleum boiling between 60 and 80 °C. The following abbreviations are used for MeCN = acetonitrile, THF = tetrahydrofuran, DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, Et_2O = diethyl ether, *p*-TSA = *p*-toluenesulfonic acid, TFA = trifluoroacetic acid, and TIS = trisisopropylsilane. All reactions were carried out in oven-dried glassware under an argon atmosphere using anhydrous solvents, a standard syringe, and septum techniques unless otherwise indicated. Organic extracts were dried over anhydrous Na_2SO_4 and then filtered prior to removal of all volatiles under reduced pressure on rotary evaporation. Chromatographic purification of products was accomplished using column chromatography on silica gels (mesh 100−200). Thin-layer chromatography (TLC) was carried out on aluminum sheets, Silica Gel 60 F254 (Merck; layer thickness = 0.25 mm). Visualization of the developed chromatogram was performed by UV light and/or phosphomolybdic acid stains. Optical rotations were measured on a Jasco P-2000 polarimeter with a sodium lamp (589 nm), a halogen lamp (880, 578, 546, 436, 405, and 325 nm), in a 10 cm cell, thermostated at 25 °C with a Peltier controlled cell holder. The electronic circular dichroism spectrum was measured on a JASCO J-815 spectrometer equipped with a JASCO Peltier cell holder PTC-423 to maintain the temperature at 25.0 ± 0.2 °C. A CD quartz cell of 1 mm of optical path length was used. IR spectra were recorded as thin films (for liquids). ^{1}H and ^{13}C NMR spectra were recorded at 300 or 500 MHz and 75 or 125 MHz, respectively, using $CDCl_3$ or CD_3OD as a solvent. Chemical shifts (δ) are given in ppm relative to the solvent residual peak or TMS as an internal standard. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were measured on a QTOF I (quadrupole-hexapole-TOF) mass spectrometer with an orthogonal Z-spray-electrospray interface on a Micro (YA−263) mass spectrometer (Manchester, UK), and matrix-assisted laser desorption ionization (MALDI) mass spectra were recorded on a Bruker ultrafleXtreme MALDI-TOF system. An automated peptide synthesizer, Biotage Initiator + Alstra, was used for the solid-phase peptide synthesis. All *Fmoc*-protected amino acids were purchased as L-enantiomers from Merck.

*Route a: N,N′-(4-Methyl-1,2-phenylene)bis(sulfanediyl)bis-(methylene)diacetamide (**3**) Using HCl.* A mixture of *N*-(hydroxymethyl)acetamide (181 mg, 2 mmol) and 98.2 mg (0.628 mmol) of **2**, in 50 mL of water, was cooled in an ice bath, and 20 mL of concentrated hydrochloric acid was added. The flask was stoppered and stirred for 1 day at room temperature under argon. After completion, the reaction mixture was concentrated *in vacuo*, and the residue was evaporated with ethanol four times. The crude product was purified by silica gel column chromatography using DCM/MeOH (95:5) to obtain product **3** (84 mg, 45%) (R_f = 0.6, DCM/MeOH, 95:5). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (d, J = 7.6 Hz, 1H), 7.22 (s, 1H), 6.99 (d, J = 7.6 Hz, 1H), 6.78 (brs, 1H), 6.66 (brs, 1H), 2.29 (s, 3H), 1.89 (s, 2H), 1.87 (s, 1H) ppm. ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 170.5, 138.8, 136.8, 133.6, 132.9, 131.9, 128.8, 43.4, 43.0, 23.0, 21.1 ppm. IR (neat): ν_{max} 3278, 3064, 29.28, 1557, 1542, 1255 cm^{-1}. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{13}H_{18}O_2N_2S_2Na$, 321.0707; found, 321.0706. MALDI-TOF: (M = $C_{13}H_{19}N_2O_2S_2$) 284.56 [M − CH_3 + H]$^+$.

*Route b: N,N′-(4-Methyl-1,2-phenylene)bis(sulfanediyl)bis-(methylene)diacetamide (**3**) Using TMS-Cl.* To a solution of *N*-(hydroxymethyl)acetamide (288 mg, 3.23 mmol) in anhydrous THF (4 mL) was added TMS-Cl (256 μL, 2.02 mmol). The solution was allowed to stir for 10 min. In a separate flask, toluene-3,4-dithiol (158.5 mg, 1.01 mmol) was dissolved in anhydrous THF (4 mL), and this solution was transferred via cannula under argon to the stirring solution and allowed to stir for 12 h. Upon completion of the reaction (monitored by TLC), the reaction mixture was quenched with water. The aqueous layer was then extracted with ethyl acetate three times, and the organic layers were combined and dried over sodium sulfate. The crude product was purified by silica gel column chromatography using DCM/MeOH (95:5) to obtain product **3** (145 mg, 48%).

*5-Methyl-1,3-benzodithiol-2-one (**4**).*[29] Compound **2** (7.55 mmol, 1.18 g, 1 mL) was dissolved in 35 mL of tetrahydrofuran. To the stirring solution, carbonyldiimidazole (7.48 mmol, 1.21 g) was added as a solid. The solution was stirred at room temperature under nitrogen, and the reaction was monitored by thin-layer chromatography (SiO_2/pentane−Et_2O 50:1). After 14 h, more carbonyldiimidazole (1.36 mmol, 220 mg) was added, and the solution stirred for 4 additional hours. The solution was then dried *in vacuo*, affording a white solid; 65 mL of 0.1 M hydrochloric acid and 60 mL of chloroform were added to the solid, and the suspension was transferred to a separatory funnel. Following phase separation, the organic phase was re-extracted with 2 × 65 mL of 0.1 M hydrochloric acid and 2 × 60 mL of water. The organic layer was then dried over Na_2SO_4 and filtered, and the solvent was removed *in vacuo*, affording the title compound as a white solid (1.265 g, 92% yield) (R_f = 0.7, Pet-ether/EtOAc, 95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (d, J = 8.1 Hz, 1H), 7.30 (s, 1H), 7.13 (d, J = 8.1 Hz, 1H), 2.39 (s, 3H) ppm. ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 190.5, 137.4, 132.7 129.4, 128.2, 123.5, 122.9, 21.4 ppm. IR (neat): ν_{max} 2926, 1740, 1693, 1647, 1452, 887, 843 cm^{-1}. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_8H_7OS_2$, 182.9933; found, 182.9930.

*5-Methyl-2,2-diphenylbenzo[d][1,3]dithiol (**5**).* Compound **2** (300 mg, 1.92 mmol), dimethoxydiphenylmethane (658 mg, 2.88 mmol), and *p*-TSA (36.5 mg, 0.192 mmol) were dissolved in dry toluene, and the reaction mixture was refluxed for 12 h in a Dean−Stark apparatus. Then toluene was removed by evaporation, and the crude mixture was dissolved in chloroform, washed with 1 N HCl, water, and brine, and dried over Na_2SO_4. The crude mixture was purified by flash column chromatography using Et_2O/Pet-ether (1:99) to give **5** as a white solid (603 mg, 98% yield) (R_f = 0.6, Pet-ether/EtOAc, 95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.66 (d, J = 7.7 Hz, 4H), 7.33−7.22 (m, 6H), 7.09 (d, J = 7.9 Hz, 1H), 7.04 (s, 1H), 6.84 (d, J = 7.8 Hz, 1H), 2.25 (s, 3H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 143.3, 138.0, 136.1, 134.6, 128.2, 128.1, 127.8, 126.9, 123.0, 122.0, 78.1, 21.1 ppm. IR (neat): ν_{max} 3055, 2918, 1489, 1457, 1443 cm^{-1}. HRMS (ESI): m/z [M]$^+$ calcd for $C_{20}H_{16}S_2$, 320.0693; found, 320.0695.

Literature Preparations. Compounds **9a$_1$**, **9b$_1$**, **9a$_2$**, **9b$_2$**, **10a**, and **10b** were prepared according to reported literature procedures.[15] Spectroscopic data of all of the compounds are in good agreement with those previously reported.

*3,4-Dimercaptobenzoic Acid (**8a**).*[15] An aqueous solution of NaOH (1 N, 10 mL) was added to compound **10a** (392 mg, 1.734 mmol). The resulting mixture was heated at 70 °C using a silicone oil bath under an argon atmosphere for 6 h. The reaction mixture was cooled to RT and acidified with 1 N HCl. The white precipitate was dissolved in EtOAc, washed several times with water, and dried over Na_2SO_4 and concentrated to afford the product **8a**, as a yellow solid (361 mg, 92%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.97 (s, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H) ppm. ^{13}C{^{1}H} NMR (75 MHz, CD_3OD): δ 168.9, 140.4, 132.9, 131.4, 130.4, 129.4, 128.4 ppm. IR (neat): ν_{max} 2924, 2854, 1681, 1586, 1314, 762 cm^{-1}.

*2,3-Dimercaptobenzoic Acid (**8b**).*[15a] Starting from compound **10b** (400 mg, 1.169 mmol), **8b** was synthesized following the procedure of **8a**. The product **8b** was obtained as a yellow solid (202 mg, 93%). ^{1}H NMR (300 MHz, CD_3OD): δ 7.86 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.02 (t, J = 7.8 Hz, 1H) ppm. ^{13}C{^{1}H} NMR (75 MHz, CD_3OD): δ 170.7, 138.5, 135.4, 133.5, 130.6, 128.7, 124.9. IR (neat): ν_{max} 2497, 1664, 1395, 1314, 1268, 744 cm^{-1}.

*2,2-Diphenylbenzo[d][1,3]dithiole-5-carboxylic Acid (**11a**).* Compound **8a**, dimethoxydiphenylmethane (474 mg, 2.081 mmol), and pTSA (30 mg, 0.173 mmol) were dissolved in 5 mL of dry toluene, and the reaction mixture was refluxed in a silicone oil bath for 12 h using a Dean−Stark apparatus. Toluene was then removed by evaporation, and the crude mixture was dissolved in chloroform, washed with 1 N HCl, water, and brine, and dried over Na_2SO_4. The crude product was purified by flash column chromatography using DCM/MeOH (98:2) to give **11a** as a white solid (509 mg, 84% yield) (R_f = 0.45, DCM/MeOH, 95:5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (s, 1H), 7.69 (dd, J = 8.1, 1.5 Hz, 1H), 7.62−7.59 (m, 4H), 7.34−7.22 (m, 7H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 167.9, 143.8, 142.6, 138.3, 128.4, 128.1, 128.0, 127.9, 127.7, 123.1, 121.6, 78.3 ppm. IR (neat): ν_{max} 3059,

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2928, 1719, 1689, 1657, 1276 cm^{-1}. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{20}H_{15}O_2S_2$, 351.0508; found, 351.0506.

*2,2-Diphenylbenzo[d][1,3]dithiole-4-carboxylic Acid (**11b**).* Starting from the compound **8b** (500 mg, 2.684 mmol), **11b** was synthesized following the procedure of **11a**. The white solid product **11b** was obtained in 81% (761 mg) yield (R_f = 0.5, DCM/MeOH, 95:5). ^1H NMR (300 MHz, $CDCl_3$): δ 7.75 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.5 Hz, 4H), 7.32 (d, J = 7.6 Hz, 1H), 7.28−7.20 (m, 6H), 7.05 (t, J = 7.70 Hz, 1H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 168.2, 143.5, 141.9, 139.5, 128.2, 128.1, 127.6, 125.6, 125.3, 124.9, 75.5 ppm. IR (neat): ν_{max} 3061, 2662, 1678, 1433, 1278, 750 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{20}H_{14}O_2S_2Na$, 373.0333; found, 373.0331.

*Methyl 2,2-Diphenylbenzo[d][1,3]dithiole-5-carboxylate (**12a**).* To a stirred solution of compound **11a** (265 mg, 0.756 mmol) in anhydrous DMF (3.5 mL) was added K_2CO_3 (209 mg, 1.51 mmol) portion wise at 0 °C. The mixture was stirred at RT for 10 min and again cooled to 0 °C, treated with CH_3I (0.188 mL, 3.024 mmol), and allowed to stir at RT for 12 h. After completion of reaction, the reaction mixture was quenched with NH_4Cl (5 mL) and extracted with EtOAc (3 × 5 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography using Pet-ether/EtOAc (95:5) on silica gel to afford a white solid **12a** (226 mg, 84% yield) (R_f = 0.5, Pet-ether/EtOAc, 90:10). ^1H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, J = 1.6 Hz, 1H), 7.35 (dd, J = 8.2, 1.6 Hz, 1H), 7.30−7.26 (m, 4H), 6.99−6.89 (m, 7H), 3.52 (s, 3H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 166.3, 144.0, 142.7, 138.6, 128.3, 128.2, 128.1, 127.6, 123.0, 121.8, 78.5, 52.2 ppm. IR (neat): ν_{max} 2950, 1717, 1582, 1430, 1285, 1234 cm^{-1}. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{17}O_2S_2$, 365.0670; found, 365.0665.

*Methyl 2,2-Diphenylbenzo[d][1,3]dithiole-4-carboxylate (**12b**).* Compound **12b** was synthesized from compound **11b** (150 mg, 0.428 mmol) following the procedure of **12a**. The isolated yield of white solid product **12b** was 81% (126 mg) (R_f = 0.5, Pet-ether/EtOAc, 90:10). ^1H NMR (300 MHz, $CDCl_3$): δ 7.33 (dd, J = 7.9, 1.1 Hz, 1H), 7.27−7.23 (m, 4H), 6.95−6.81 (m, 7H), 6.65 (t, J = 7.8 Hz, 1H), 3.50 (s, 3H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 166.4, 143.5, 141.8, 139.7, 128.2, 128.1, 127.7, 127.6, 125.5, 125.4, 124.5, 75.6, 52.5 ppm. IR (neat): ν_{max} 3058, 2949, 1705, 1443, 1403, 1301, 1270, 746 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{21}H_{16}O_2S_2Na$, 387.0490; found, 387.0492.

*(2,2-Diphenylbenzo[d][1,3]dithiol-5-yl)methanol (**13a**).* Anhydrous $AlCl_3$ (285.19 mg, 2.14 mmol) was added portion wise to a suspension of $LiAlH_4$ (270.58 mg, 7.13 mmol) in 5 mL of anhydrous Et_2O under an argon atmosphere at 0 °C. The reaction mixture was stirred at the same temperature for 5 min, and the ester compound **12a** (260 mg, 0.71 mmol) dissolved in 8 mL of anhydrous Et_2O was cannula transferred to the stirring solution over a period of 10 min. The resulting mixture was continuously stirred at 0 °C for 5 min and then quenched with 0.1 N HCl and extracted with Et_2O (3 × 5 mL). The combined organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography using Pet-ether/EtOAc (4:1) on silica gel to afford the alcohol **13a** as a colorless oil (220 mg, 92% yield) (R_f = 0.5, Pet-ether/EtOAc, 85:15). ^1H NMR (300 MHz, $CDCl_3$): δ 7.65−7.57 (m, 4H), 7.34−7.20 (m, 6H), 7.16−7.12 (m, 1H), 7.04 (d, J = 7.8 Hz, 0.5H), 6.95 (d, J = 10.2 Hz, 1H), 6.82 (d, J = 7.88 Hz, 0.5H), 4.51 (d, J = 12.7 Hz, 2H), 1.89 (s, 1H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 155.0, 143.1, 142.9, 139.6, 139.1, 138.4, 137.2, 128.5, 128.2, 127.9, 126.9, 124.9, 122.2, 121.6, 121.4, 120.9, 109.7, 78.2, 65.0, 64.7 ppm. IR (neat): ν_{max} 3404, 2950, 1431, 1289, 1240 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{20}H_{16}OS_2Na$, 359.0540; found, 359.0542.

*(2,2-Diphenylbenzo[d][1,3]dithiol-4-yl)methanol (**13b**).* Starting from compound **12b** (254 mg, 0.697 mmol), **13b** was synthesized following the procedure of **13a**. The isolated yield of the oily colorless product **13b** was 91% (213 mg) (R_f = 0.5, Pet-ether/EtOAc, 85:15). ^1H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, J = 7.0 Hz, 4H), 7.35−7.28 (m, 6H), 7.19 (dd, J = 7.4, 1.1 Hz, 1H), 7.10−7.03 (m, 2H), 4.59 (s, 3H), 2.15 (s, 1H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 143.1, 138.6, 136.4, 135.2, 128.1, 127.9, 126.1, 124.9, 121.5, 77.8, 64.7 ppm. IR (neat): ν_{max} 3363, 3058, 2870, 1489, 1443, 1249, 753, 695 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{20}H_{16}OS_2Na$, 359.0540; found, 359.0541.

*5-(Bromomethyl)-2, 2-diphenylbenzo[d][1,3]dithiol (**7a**). Route A.* To an ice-cold solution of PBr_3 (0.055 mL, 0.588 mmol) in 4 mL of anhydrous Et_2O under argon was added dropwise a solution of compound **13a** (153 mg, 0.59 mmol) in 6 mL of anhydrous Et_2O, and the mixture was allowed to stir at 0 °C for 4 h. After completion of the reaction (TLC monitoring), the solvent was evaporated, and the reaction mixture was diluted with EtOAc (15 mL). The combined organic layers were washed with water, followed by brine. Drying over Na_2SO_4 and evaporation of the solvent under reduced pressure afforded the product as a white solid (160 mg, 85% yield), which was used directly in the next reaction without further purification.

Route B. To a stirred solution of compound **5** (1.00 g, 3.12 mmol) in anhydrous CH_2Cl_2 (20 mL) was added *N*-bromosuccinimide (555 mg, 3.11 mmol). The solution was irradiated and heated to reflux with a halogen lamp (500 W) for 30 min. At this point, more *N*-bromosuccinimide (111 mg, 0.62 mmol) was added, and the solution was irradiated and heated to reflux with the halogen lamp (500 W) for an additional hour. The reaction mixture was added to a separatory funnel and extracted with 4 × 150 mL of a 0.1 M $NaHCO_3$ solution and then with 1 × 150 mL water. Drying over Na_2SO_4 and evaporation of the solvent under reduced pressure afforded the product as an off-white powder. The crude product was recrystallized 2-fold from pentane to afford **7a** as a white crystalline solid (814 mg, 65% yield) (R_f = 0.7, Pet-ether/EtOAc, 95:5). ^1H NMR (300 MHz, $CDCl_3$): δ 7.58−7.54 (m, 4H), 7.26−7.18 (m, 6H), 7.15 (d, J = 1.8 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 6.97 (dd, J = 8.1, 1.8 Hz, 1H), 4.31 (s, 2H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$):δ = 143.0, 138.8, 138.5, 135.9, 128.3, 128.0, 127.0, 122.8, 122.3, 78.4, 33.1 ppm. IR (neat): ν_{max} 2960, 2922, 1633, 1423, 889, 690 cm^{-1}. HRMS (ESI):[30] m/z $[M + Na]^+$ calcd for $C_{20}H_{15}BrS_2Na$, 420.9696; found, 420.9699. The structure was deposited on the CCDC (CCDC 2035076)

*4-(Bromomethyl)-2,2-diphenylbenzo[d][1,3]dithiol (**7b**).* Starting from **13b** (200 mg, 0.594 mmol), **7b** was synthesized following the procedure of **7a** via route A. Product **7b** was obtained as a white crystalline solid in 85% (202 mg) yield (R_f = 0.6, Pet-ether/EtOAc, 95:5). ^1H NMR (300 MHz, $CDCl_3$): δ 7.68−7.57 (m, 4H), 7.35−7.21 (m, 6H), 7.12 (dd, J = 6.9, 2.1 Hz, 1H), 7.01−6.93 (m, 2H), 6.87 (m, 1H), 4.42 (s, 1.25H), 4.35 (s, 0.75H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 155.4, 142.8, 142.7, 139.5, 138.6, 132.0, 131.2, 128.6, 128.3, 128.0, 127.3, 127.2, 127.0, 126.5, 126.4, 123.5, 122.5, 110.9, 78.2, 33.2, 32.6 ppm. IR (neat): ν_{max} 3058, 1489, 1442, 1412, 753, 695 cm^{-1}. HRMS (ESI): m/z ($[M - Br + Na]^+$ 100%) calcd for $C_{20}H_{15}S_2Na$, 342.0513; found, 342.0515. The structure was deposited on the CCDC (CCDC 1840432).

*Diethyl 2-((2,2-Diphenylbenzo[d][1,3]dithiol-5-yl)methyl)-2-(2,2,2-trifluoroacetamido)malonate (**6a**).* To a stirred solution of trifluoroacetamido malonate **16** (152 mg, 0.56 mol) in 2.5 mL of anhydrous DMF was added NaH in 60% mineral oil (26 mg, 0.67 mmol) portion wise at 0 °C. After being stirred at RT for 20 min, the resulting mixture was further cooled to 0 °C. The solution of bromide **7a** (224 mg, 0.56 mmol) in 2 mL of anhydrous DMF was then transferred to the solution via cannula under argon at the same temperature. After the addition was completed, the reaction mixture was slowly warmed to RT, and stirring was continued at the same temperature for 6 h. After cooling to 0 °C, the reaction mixture was diluted with EtOAc (3 × 5 mL), washed successively with 0.1 N HCl, water, and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography using ethyl acetate−petroleum ether (1:9) on silica gel to afford **6a** as a light yellowish gummy product (270 mg, 82% yield) (R_f = 0.5, Pet-ether/EtOAc, 80:20). ^1H NMR (400 MHz, $CDCl_3$): δ 7.62−7.55 (m, 4H), 7.41 (s, 1H), 7.35−7.22 (m, 6H), 7.04 (dd, J = 39.6, 7.9 Hz, 1H), 6.84 (d, J = 1.2 Hz, 1H), 6.62 (m, 1H), 6.50 (dd, J = 7.8, 1.3 Hz, 1H), 4.35−4.19 (m, 4H), 3.59 (d,J = 11.9 Hz, 2H), 1.27 (q, J = 7.1 Hz, 6H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 166.1, 156.1 (q, 1C, J = 38 Hz), 155.0, 143.0, 142.8, 138.7, 137.6, 132.2, 132.0, 128.6, 128.1, 128.0, 127.2, 126.9, 125.9, 123.9, 123.3, 122.4, 121.7, 119.7, 116.8, 113.9, 112.2, 111.1, 104.1, 78.4, 67.4,

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67.3, 63.5, 63.5, 37.1, 36.9, 14.0 ppm. IR (neat): ν_{max} 3392, 2918, 1729, 1524, 1443, 1278, 1168, 1002, 753, 701 cm^{-1}. HRMS (ESI): m/z $[M + K]^+$ calcd for $C_{29}H_{26}F_3NO_5S_2K$, 628.0842; found, 628.0844.

*Diethyl 2-((2,2-Diphenylbenzo[d][1,3]dithiol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)malonate (**6b**).* Compound **6b** was synthesized from compound **7b** (189 mg, 0.473 mmol), following the same procedure of **6a**. Compound **6b** was obtained as a light yellowish gummy product. Yield: 75% (209 mg) (R_f = 0.5, Pet-ether/EtOAc, 80:20). ^1H NMR (400 MHz, $CDCl_3$): δ 7.55−7.53 (m, 5H), 7.37−7.27 (m, 6H), 7.15 (d, J = 8 Hz, 1H), 7.0−6.9 (m, 1H), 6.7 (d, J = 7.6 Hz, 1H), 4.37−4.29 (m, 2H), 4.25−4.16 (m, 2H), 3.73 (s, 2H), 1.28 (t, J = 7.2 Hz, 6H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 166.14, 166.11, 156.4 (q, 1C, J = 38 Hz), 155.1, 143.0, 142.9, 139.6, 138.9, 128.7, 128.6, 128.2, 128.16, 128.03, 127.97, 126.9, 126.3, 126.0, 124.3, 121.6, 116.8, 114.0, 110.2, 103.5, 66.9, 63.59, 63.55, 38.4, 37.5, 13.9 ppm. IR (neat): ν_{max} = 3386, 3055, 2984, 1729, 1519, 1444, 1216, 1167, 696 cm^{-1}. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{29}H_{27}F_3NO_5S_2$, 590.1277; found, 590.1276.

*Diethyl 2-Acetamido-2-((2-phenylbenzo[d][1,3]dithiol-5-yl)-methyl)malonate (**17a**).* Compound **17a** was synthesized from **7a** (170 mg, 0.425 mmol) and malonate derivative **14**, following the same procedure of **6a**, and was obtained as a white solid product. Yield: 65% (148 mg) (R_f = 0.5, Pet-ether/EtOAc, 80:20). ^1H NMR (300 MHz, $CDCl_3$): δ 7.53 (dd, J = 8.4, 1.8 Hz, 4H), 7.24−7.14(m, 6H), 7.0 (d, J = 5.4 Hz, 1H), 6,78 (s, 1H), 6.58 (dd, J = 7.8, 1.2 Hz, 1H), 6.47 (s, 1H), 4.23−4.10 (m, 4H), 3.47 (s, 2H), 1.91(s, 3H), 1.17 (t, J = 7.2 Hz, 6H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 169.2, 167.4, 143.1, 138.2, 136.9, 133.5, 128.2, 128.0, 127.6, 123.6, 122.1, 78.3, 67.2, 62.8, 37.5, 23.1, 14.1 ppm. IR (neat): ν_{max} 3405, 3064, 2983, 1740, 1680, 1493, 1443, 1276, 1198, 696 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{29}H_{29}NO_5S_2Na$, 558.1385; found, 558.1387.

*Diethyl 2-(tert-Butoxycarbonylamino)-2-((2,2-diphenylbenzo[d]-[1,3]dithiol-5-yl)methyl)malonate (**18a**).* Compound **18a** was synthesized from **7a** (165 mg, 0.413 mmol) and malonate derivative **15**, following the same procedure of **6a**. A colorless oily product was obtained. Yield: 32% (82 mg) (R_f = 0.5, Pet-ether/EtOAc, 80:20). ^1H NMR (300 MHz, $CDCl_3$): δ 7.62 (dd, J = 7.8, 1.5 Hz, 4H), 7.32−7.24 (m, 6H), 7.07 (d, J = 8.1 Hz, 1H), 6.90 (s, 1H), 6.70 (d, J = 7.8 Hz, 1H), 5.74 (s, 1H), 4.29−4.12 (m, 4H), 3.52 (s, 2H), 1.46 (s, 9H), 1.24 (t, J = 7.2 Hz, 6H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 167.6, 154.0, 143.2, 138.1, 136.7, 133.6, 128.20, 128.18, 128.1, 127.9, 127.6, 123.9, 122.0, 80.4, 78.2, 67.2, 62.7, 38.0, 28.4, 14.1 ppm. IR (neat): ν_{max} 3431, 2979, 1740, 1715, 1489 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{32}H_{35}NO_6S_2Na$, 616.1804; found, 616.1805.

*2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(2,2-diphenylbenzo[d][1,3]dithiol-5-yl)propanoic Acid (**19a**).* To an ice-cold solution of malonate derivative **6a** (247 mg, 0.42 mmol) in THF/H_2O (1:1) (10 mL) was added LiOH·H_2O (120.38 mg, 5.016 mmol), and the mixture was allowed to reflux for 12 h in a silicone oil bath. After completion of reaction (TLC monitoring), THF was removed, the remaining aqueous phase was first cooled to 0 °C, acidified to pH 2 with 0.1 N HCl, and then neutralized by adding solid $NaHCO_3$ and diluted with 5 mL of dioxane. A solution of *Fmoc*-OSu (282 mg, 0.84 mmol) in 7 mL of dioxane was then added dropwise to the suspension at the same temperature, and the resulting mixture was allowed to stir at RT for 12 h. When the reaction was completed, the solvent was evaporated, and the remaining aqueous phase was further acidified with 0.1 N HCl and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with water, followed by brine, dried over Na_2SO_4, and concentrated to afford the product **19a** as a white foamy solid (194 mg, 75% yield) (R_f = 0.5, DCM/MeOH, 92:8), which was used for the esterification reaction without further purification. ^1H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J = 7.5 Hz, 2H), 7.608−7.577 (m, 4H), 7.52 (d, J = 6.6 Hz, 2H), 7.37 (t, J = 7.2 Hz, 2H), 7.30−7.19 (m, 8H), 7.06 (d, J = 7.8 Hz, 2H), 6.98 (s, 1H), 5.29 (d, J = 8.1 Hz, 1H), 4.61 (d, J = 6.3 Hz, 1H), 4.43−4.29 (m, 2H), 4.19−4.15 (m, 1H), 3.09−2.93 (m, 2H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$):δ = 175.6, 155.9, 143.8, 143.7, 143.1, 141.4, 138.5, 136.8, 133.9, 127.8, 127.2, 125.2, 125.1, 123.0, 122.3, 120.1, 78.2, 67.2, 54.6, 47.2, 37.4 ppm. IR (neat): ν_{max} 3273, 1737, 1699, 1655, 1450, 891 cm^{-1}. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{37}H_{30}NO_4S_2$, 616.1611; found, 616.1612.

*2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(2,2-diphenylbenzo-[d][1,3]dithiol-4-yl)propanoic Acid (**19b**).* Starting from **6b**, **19b** was synthesized following the procedure of **19a**. Compound **19b** was obtained as a white foamy solid product. Yield: 72% (R_f = 0.5, DCM/MeOH, 92:8). ^1H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, J = 7.5 Hz, 2H), 7.60 (t, J = 8.8 Hz, 4H), 7.48 (d, J = 7.2 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 7.23−7.13 (m, 8H), 7.07 (d, J = 7.5 Hz, 1H), 6.91 (t, J = 7.5 Hz, 1H), 6.84(m, 1H), 5.34 (d, J = 7.5 Hz, 1H), 4.68 (d, J = 5.4 Hz, 1H), 4.36−4.23 (m, 2H), 4.11 (m, 1H), 3.26−3.01 (m, 2H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 175.8, 156.0, 143.9, 143.7, 143.2, 142.6, 141.3, 138.6, 130.8, 128.1, 127.9, 127.9, 127.8, 127.4, 127.2, 126.4, 125.3, 125.2, 121.2, 120.0, 77.3, 67.4, 54.0, 47.1, 38.1 ppm. IR (neat): ν_{max} 3068, 2928, 1714, 1508, 1447, 740 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{37}H_{29}NO_4S_2Na$, 638.1436; found, 638.1438.

*Methyl-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(2,2-diphenylbenzo[d][1,3]dithiol-5-yl)propanoate (**1a**).* To a suspended solution of compound **19a** (212 mg, 0.34 mmol) in anhydrous DMF (3 mL) was added K_2CO_3 (94 mg, 0.68 mmol) portion wise at 0 °C. The mixture was stirred at RT for 10 min and again cooled to 0 °C, treated with CH_3I (0.084 mL, 1.36 mmol), and allowed to stir at RT for 12 h. After completion of reaction, the reaction mixture was quenched with NH_4Cl (5 mL) and extracted with EtOAc (3 × 5 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography using ethyl acetate−petroleum ether (1:6) on silica gel to afford **1a** as a white solid (167 mg, 78% yield) (R_f = 0.5, Pet-ether/EtOAc, 75:25). ^1H NMR (300 MHz, $CDCl_3$): δ 7.77 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 10.8 Hz, 4H), 7.57 (m, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.35−7.09 (m, 8H), 7.11 (d, J = 7.8 Hz, 1H), 6.96 (s, 1H), 6.75 (d, J = 7.8 Hz, 1H), 5.24 (d, J = 8.1 Hz, 1H), 4.61 (m, 1H), 4.47−4.32 (m, 2H), 4.21 (m, 1H), 3.65 (s, 3H), 2.99 (d, J = 5.7 Hz, 2H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$):δ = 171.8, 155.6, 143.9, 143.8, 143.1, 141.4, 138.5, 136.8, 134.0, 128.5, 128.2, 128.2, 127.9, 127.8, 127.2, 127.1, 127.0, 126.9, 125.2, 125.1, 123.7, 123.0, 120.1, 67.1, 54.8, 52.4, 47.3, 38.0 ppm. IR (neat): ν_{max} 3333, 3064, 2951, 1723, 1521, 1445, 1213, 740 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{38}H_{31}NO_4S_2Na$, 652.1592; found, 652.1593.

*Methyl 2-(((9H-Fluoren-9-yl) methoxy) carbonylamino)-3-(2,2-diphenylbenzo[d][1,3]dithiol-4-yl)propanoate (**1b**).* Starting from compound **19b** (100 mg, 0.162 mmol), **1b** was synthesized following the procedure of **1a**. Compound **1b** was obtained as a white solid product: yield 77% (78 mg) (R_f = 0.5, Pet-ether/EtOAc, 75:25). ^1H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, J = 7.5 Hz, 2H), 7.66−7.60 (m, 4H), 7.54 (d, J = 7.2 Hz, 2H), 7.39 (m, 2H), 7.30−7.18 (m, 8H), 7.12 (d, J = 7.5 Hz, 1H), 6.97 (m, 1H), 6.82 (d, J = 7.5 Hz, 1H), 5.34 (d, J = 7.2 Hz, 1H), 4.72 (q, J = 7.0 Hz, 1H), 4.41−4.15 (m, 2H), 4.17 (m, 1H), 3.69 (s, 3H), 3.22−3.04 (m, 2H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ 172.0, 155.7, 144.0, 143.8, 143.3, 142.7, 141.3, 138.7, 138.6, 130.7, 128.5, 128.2, 128.2, 127.9, 127.8, 127.3, 127.2, 126.9, 126.3, 125.3, 125.2, 121.2, 120.0, 67.2, 54.0, 52.6, 47.2, 38.7 ppm. IR (neat): ν_{max} 3418, 3068, 2925, 1723, 1521, 1447, 740 cm^{-1}. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{38}H_{31}NO_4S_2Na$, 652.1592; found, 652.1593.

Optical Resolution by Preparative Chiral HPLC. Chiral amino acids **1a** and **1b** were analyzed and purified on Chiralpak IF and Chiralpak ID columns. The two fractions collected were analyzed by analytical chiral HPLC, a polarimeter, and ECD and VCD.

*Chromatographic Conditions for **1a**.* Compound **1a** was prepurified on a preparative Chiralpak ID to remove ∼10% of unidentified impurities. Then 380 mg of the purified racemic compound was dissolved in 8 mL of a mixture of CH_2Cl_2/ethanol (1:1). It was injected on a Chiralpak IF column (250 mm × 10 mm) in 160 × 50 μL stacked injections (every 4.2 min), using hexane/*i*PrOH/DCM (80:10:10) as a mobile phase with a flow rate of 5 mL/min, and detected with an UV detector at 280 nm. Two fractions were collected and analyzed on an analytical Chiralpak IF column using heptane/*i*PrOH/DCM (80:10:10) as an isocratic eluant. Compound **1a**-*R*: 147 mg, t_R = 9.8 (ee > 99.5%), $[\alpha]_D^{25}$ ($CHCl_3$, c = 0.89) = −29. Crystals could be grown by slow evaporation at 4 °C of a solution containing 110

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mg of the first eluted enantiomer for **1a** in CH_2Cl_2/EtOH (1:1) in the presence of traces of pentane and water. The structure was deposited on the CCDC (CCDC 2035077). Compound **1a**-*S*: 139 mg, t_R = 11.48 min (ee > 99.5%), $[\alpha]_D^{25}$ ($CHCl_3$, c = 0.90) = +29. Crystals could be grown in similar conditions as those for **1a**-*R*; however, the needles were smaller, and the structure could not be solved.

*Chromatographic Conditions for **1b**.* About 320 mg of racemic compound **1b** was dissolved in 2 mL of DCM, injected on Chiralpak ID (250 mm × 10 mm) in 40 × 50 μL stacked injections (every 3.8 min), using hexane/*i*PrOH/DCM (70:10:20) as a mobile phase with a flow rate of 5 mL/min, and detected with an UV detector at 290 nm. Two fractions were collected and analyzed on analytical Chiralpak ID using Heptane/*i*PrOH/CH_2Cl_2 (70:10:20) as an isocratic eluant. (1) Unidentified: 145 mg, t_R = 5.51 min (ee > 99%), $[\alpha]_D^{25}$ ($CHCl_3$, c = 1.16) = +10.5. (2) Unidentified: 135 mg, t_R = 6.48 min (ee > 99.5%), $[\alpha]_D^{25}$ ($CHCl_3$, c = 0.95) = −10.5.

*2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(2,2-diphenylbenzo[d][1,3]dithiol-5-yl)propanoic Acid (**19a**).* Compound **1a** (84 mg, 0.133 mmol) was dissolved in isopropanol (2.5 mL) and THF (0.8 mL). $CaCl_2$ (237 g, 2.13 mmol) was added. Separately, LiOH·H_2O (22.4 mg, 0.533 mmol) was dissolved in H_2O (1.1 mL). The aqueous solution was then added to the reaction mixture, and the cloudy white solution was stirred for 2.5 h. The organic solvents were removed under reduced pressure, and the resulting residue was taken up in 10% potassium carbonate (K_2CO_3) (5 mL) as a cloudy white suspension. The aqueous layer was partitioned in Et_2O (2 × 5 mL) to remove the *Fmoc* deprotection side products (if any), after which it was acidified to pH 2 with dilute HCl. It was then extracted with EtOAc (30 mL). The organic layers were dried over Na_2SO_4 and concentrated to obtain **19a** as a white foamy solid in 98% yield (80.4 mg, 0.131 mmol). The ^{1}H NMR spectrum recorded on the product matched that for **19a** reported above. This method was also used to deprotect single enantiomers **1a**-*R* and **1a**-*S*, respectively, leading to enantiopure **19a**-*R* and **19a**-*S*.

*2-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-3-(2,2-diphenylbenzo-[d][1,3]dithiol-4-yl)propanoic Acid (**19b**).* Compound **19b** was obtained from **1b** (91 mg, 0.144 mmol) in 90% yield (80 mg, 0.130 mmol). The procedure was the same as the synthesis of **19a**.

*Methyl 2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(3,4-dimercaptophenyl)propanoate (**21a**).* HBF_4 (35 wt %) in H_2O (0.3 mL, 1.225 mmol) was added at RT to a suspension of mercury(II) oxide (60 mg, 0.277 mmol) in freshly distilled THF (2 mL). Compound **19a** (110 mg, 0.175 mmol) was dissolved in THF (3 mL), and it was added to the stirring reaction mixture, in one portion. The reaction was moderately exothermic, and the color of the solution changed from an orange to colorless solution. After completion of the reaction (TLC monitoring), THF was evaporated to afford a sticky material. The addition of water (3 × 25 μL) to this crude mixture followed by decantation yielded a precipitate. The precipitate was then washed with Pet-ether/EtOAc (90:10), and the organic layer was decanted. The trituration procedure was repeated until the benzophenone was completely removed (TLC monitoring in Pet-ether/EtOAc, 95:5) and dried *in vacuo* to get a pale yellow solid. The solid was suspended in dry EtOAc (5 mL) in a strictly anaerobic atmosphere (Schlenk line), and H_2S (g) (Caution! H_2S is a highly toxic compound and should be handled with care) was flown over for 4 h, imparting a color change from yellow to orange then black (a black precipitate formed over the course of the initial 15 min) (Figure S17). The suspension was then centrifuged. The supernatant solution was separated by decantation, and the residual solid particles were further washed with MeCN and centrifuged two or three times. Finally, the collected supernatants were combined and dried *in vacuo* to get a yellowish solid product, which was directly submitted for characterization. The isolated yield of **21a** is 44% (36 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83−7.67 (m, 2H), 7.65−7.46 (m, 2H), 7.45−7.03 (m, 6H), 6.94−6.65 (m, 1H), 5.63−5.18 (m, 1H), 4.74−4.52 (m, 1H), 4.50−4.28 (m, 2H), 4.27−4.10 (m, 1H), 3.83−3.54 (m, 3H), 3.18−2.73 (m, 2H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 171.6, 155.5, 143.7, 143.6, 141.2, 134.7, 131.7, 131.6, 131.4, 129.6, 127.7, 127.0, 124.9, 119.9, 66.9, 54.5, 52.4, 47.1, 37.5 ppm. IR (neat): ν_{max} 3016, 2951, 2555, 1708, 1505, 1448, 1343, 1213, 1105, 1054, 753 cm^{-1}. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{25}H_{23}NO_4S_2Na$, 488.0966; found, 488.0964.

*Methyl 2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2,3-dimercaptophenyl)propanoate (**21b**).* Starting from compound **19b** (110 mg, 0.175 mmol), **21b** was synthesized following the procedure of **21a**. The isolated yield of **21b** was 43% (35 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (br s, 2H), 7.55 (br s, 2H), 7.45−7.14 (m, 5H), 7.13−6.92 (m, 1H), 6.91−6.62 (m, 1H), 5.44 (br s, 1H), 4.90−4.57 (m, 1H), 4.47−4.23 (m, 2H), 4.24−4.04 (m, 1H), 3.73 (s, 3H), 3.53−3.24 (m, 1H), 3.24−2.87 (m, 1H) ppm. ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): δ 172.2, 155.6, 143.7, 141.2, 139.3, 129.8, 128.7, 127.7, 127.0, 126.3, 125.0, 122.4, 119.9, 113.8, 67.0, 54.1, 52.4, 47.0, 37.5 ppm. IR (neat): ν_{max} 3334, 3017, 2952, 2549, 1719, 1699, 1576, 1514, 1477, 1462, 1446, 1339, 1213, 1104, 1051, 752 cm^{-1}. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{25}H_{23}NO_4S_2Na$, 488.0966; found, 488.0965.

*Synthesis of H_2N-Tyr-Leu-Ser-Ala-Phe-His-Ala-Glu−Phe$^{3,4\text{-dt}}$(CPh_2)-Gly-$CONH_2$ (**23**).* The linear sequence *Fmoc*-Tyr(O^tBu)-Leu-Ser(O^tBu)-Ala-Phe-His(Trt)-Ala-Glu(O^tBu)-L-Phe$^{3,4\text{-dt}}$(CPh_2)-Gly-resin was assembled on a rink amide resin (0.1 mmol, 0.59 mmol/g, 169 mg) by mixed manual and automated solid-phase peptide synthesis. The resin was preswollen in DMF at room temperature for 30 min. The *Fmoc*-deprotections were performed on the synthesizer at room temperature in two successive steps of 3 and 10 min, each involving 4.5 mL of 20% piperidine in DMF. The first peptide coupling (Gly) was performed on the automated microwave synthesizer at 75 °C for 5 min after the addition of *Fmoc*-Gly-COOH (148 mg, 0.5 mmol) in DMF (1.72 mL), HBTU (186 mg, 0.49 mmol) in DMF (0.82 mL), and DIEA (174 μL, 1.0 mmol) in NMP (0.5 mL). The second coupling (L-Phe$^{3,4\text{-dt}}$) was performed manually at 25 °C for 60 min after the addition of 2 equiv of *Fmoc*-L-Phe$^{3,4\text{-dt}}$(CPh_2)-COOH, **19a**-*S*, (0.2 mmol, 123 mg) in DMF (0.8 mL), HBTU (76 mg, 0.2 mmol) in DMF (0.33 mL), and DIEA (67 μL, 0.4 mmol) in NMP (0.2 mL). A Kaiser test indicated that the reaction did not reach completion, and this coupling step was repeated using an extra 1.1 equiv of **19a**-*S* (the same protocol as before). The following coupling steps were performed on the automated microwave synthesizer following the same protocol as for Gly, except the His coupling, which was performed at 50 °C for 6.5 min. The final peptide was first *Fmoc*-deprotected and afterward simultaneously cleaved from the resin and side-chain deprotection (except for the dithioketal function). For this, the aliquot of resin was treated with 2 × 5 mL of 20% piperidine in DMF for 10 min. After each treatment, the solution was filtered out, and then the resin was successively washed with 5 × 5 mL of DMF and 5 × 5 mL of DCM. Then, the resin was treated with 5 mL of TFA/TIS/water (95:2.5:2.5) cocktail for 2 h. The volume of the solution was decreased by flushing N_2 at the top of the solution. The crude peptide was precipitated by the addition of cold Et_2O and further decanted by centrifugation. The solid was triturated in cold diethyl ether and decanted by centrifugation twice. The peptide was redissolved in a water/acetonitrile/TFA (50:50:0.1) mixture and lyophilized to afford an off-white feather-like solid product (60 mg, 44%). ^{1}H NMR (300 MHz, D_2O/CD_3OD (1:1), water presaturation): δ 8.69 (s, 1H), 7.55 (m, 4 H), 7.34−7.25 (m, 7H), 7.25−7.07 (m, 9H), 6.97 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 8.2 Hz, 2H), 4.63−4.41 (m, 4H), 4.33−4.13 (m, 4H), 4.04−3.68 (m, 5H), 3.30−2.94 (m, 7H), 2.78 (m, 1H), 2.21 (m, 2H), 1.83 (m, 2H), 1.63 (m, 3H), 1.37 (d, J = 7.2 Hz, 3H), 1.32 (d, J = 7.2 Hz, 3H), 0.91 (m, 6H). HRMS (ESI): m/z [M + 2H]$^{2+}$ calcd for $C_{68}H_{83}N_{13}O_{14}S_2$, 684.7806; found, 684.7803.

*Synthesis of H_2N-Tyr-Leu-Ser-Ala-Phe-His-Ala-Glu−Phe$^{3,4\text{-dt}}$-Gly-$CONH_2$ (**24**).* The peptide **23** (10.4 mg, 7 μmol) dissolved in 1 mL of dry THF was treated with HBF_4 (35 wt %) in H_2O (12 μL, 0.047 mmol), followed by the addition of mercury(II) oxide (2.27 mg, 0.01 mmol), and was stirred for 2 h. THF was evaporated to dryness. Water (15 μL) was then added, and the aqueous layer was decanted carefully, affording a pale yellow precipitate. Next, the precipitate was washed with Pet-ether/EtOAC (90:10) to remove the benzophenone side product and dried *in vacuo*. The solid mass was suspended in dry EtOAc/MeOH (1.5 mL using ratio 95:5) under an argon balloon, and H_2S was flown for 15 min. The reaction mixture was stirred for an additional 4 h, resulting in the formation of a black precipitate. The

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mixture was then centrifuged and filtered. The black precipitate was washed again with EtOAc/MeOH (2 × 1.5 mL using ratio 95:5). The filtered solution was removed *in vacuo* to obtain a black solid peptide. The methanolic solution of the peptide was then dialyzed in the presence of DTT (3 equiv) for 48 h to get a white solid product **24** (3.1 mg, 37%). Analytical RP-HPLC (SPOLAR C18 S5 column, 0.8 mL min^{-1}, gradient 50−80% B in A over 25 min with, A, water + 0.1% TFA and, B, water/MeCN (10:90) + 0.1% TFA) t_R = 4.5 min. IR (neat): ν_{max} 3332, 2948, 2535, 1695, 1577, 1477, 1462, 1410, 1336, 1225, 1050, 1019, 739 cm^{-1}. MALDI-TOF MS: m/z $[M + Na]^+$ calcd for $C_{55}H_{73}N_{13}O_{14}S_2Na$, 1226.4739; found, 1230.220. MALDI-TOF MS: m/z $[M + K]^+$ calcd for $C_{55}H_{73}N_{13}O_{14}S_2K$, 1242.4478; found, 1247.521. HRMS (ESI): m/z $[M + H]^+$ calcd for dithiete compound $C_{55}H_{72}N_{13}O_{14}S_2$, 1202.4757; found, 1202.4755.

Vibrational Circular Dichroism. Infrared (IR) and vibrational circular dichroism (VCD) spectra were recorded on a Bruker PMA 50 accessory coupled to a Vertex70 Fourier transform infrared spectrometer. A photoelastic modulator (Hinds PEM 90) set at l/4 retardation was used to modulate the handedness of the circularly polarized light at 50 kHz. Demodulation was performed by a lock-in amplifier (SR830 DSP). An optical low-pass filter (<1800 cm^{-1}) before the photoelastic modulator was used to enhance the signal/noise ratio. A transmission cell equipped with BaF_2 windows and 200 μm of optical path length was used. Solutions with a concentration of 0.07 mol L^{-1} (for the enantiomers of **1a**) and 0.05 mol L^{-1} (for the enantiomers of **1b**) were prepared by dissolving the solid samples in CD_2Cl_2. The VCD spectra of the pure enantiomers of (first eluted)-**1a** and (second eluted)-**1a**, on one hand, and of (first eluted)-**1b** and (second eluted)-**1b**, on the other hand, were measured at RT, and the baseline of the spectra was corrected using the standard procedure of the half-subtraction of the spectra of each enantiomer. For each individual spectrum, about 12 000 scans were averaged at 4 cm^{-1} resolution (corresponding to a 3 h measurement time). For IR absorption spectra, the cell filled with CD_2Cl_2 served as a reference. The spectra are presented without smoothing and further data processing.

Computational Details for the Calculations of the IR/VCD Spectra. Prior to the calculations of the IR and VCD spectra, a conformational analysis has been performed using a stochastic exploration (annealing) of the potential energy surface (SEP) of the (*R*) enantiomer of **1a** and of **1b**. The annealing was done at the semiempirical level PM3, starting from a geometry optimized using density functional theory (DFT) with a B3LYP functional and 6-311G(d,p) basis set and including solvent (CD_2Cl_2) effects with a polarizable continuum model (SMD). During the annealing, only the dihedral angles have been relaxed, and the bond lengths and the valence angles were kept frozen. This step allowed us to find 69 and 65 conformers, respectively, for **1a**-*R* and **1b**-*R*, among which the 30 and 20 most stable have been fully optimized using the SMD (CD_2Cl_2)/B3LYP/6-311G (d,p) level. Using the geometries that have converged, we calculated the Boltzmann population in CD_2Cl_2 in Table S7 for **1a**-*R* and **S8** for **1b**-*R*. For the construction of averaged IR and VCD spectra, only conformations with a Boltzmann population >3% have been used. The vibrational frequencies, IR absorption, and VCD intensities were calculated using the same theoretical level as for geometry optimization SMD(CH_2Cl_2)/B3LYP/6-311G(d,p). Computed harmonic frequencies have been calibrated using a standard scaling factor of 0.98. Indeed, because of the incomplete treatment of electron correlation, harmonic approximation, and basis set truncation effects, the calculated frequencies generally overestimate. In order to improve the agreement between the calculated and measured frequencies, the computed harmonic frequencies are usually scaled for comparison. IR absorption and VCD averaged spectra were constructed from calculated dipole and rotational strengths, assuming the Lorentzian band shape with a half-width at half-maximum of 8 cm^{-1}. All calculations were performed using Ampac10[31] (simulated annealing) and the Gaussian 16 package.[32]

General Procedure for XPS Analysis. The X-ray photoemission spectroscopy (XPS) measurements were carried out using an Omicron electron spectrometer, equipped with a Scienta omicron sphera analyzer and Al Kα monochromatic source with an energy resolution of 0.5 eV. The samples were prepared on Si surfaces. Before collecting the spectra, the sample surface was sputtered with argon ion bombardment for each of these samples to remove any kind of surface oxidization effect and the presence of environmental carbons in the pelletized samples.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02359.

Copies of NMR (^1H, ^{13}C) spectra of all the new compounds along with X-ray structure, IR, VCD, and CD spectra, HPLC chromatogram, and HRMS data of relevant compounds (PDF)

Cartesian coordinates (PDF)

Accession Codes

CCDC 1840432 and 2035076−2035077 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

■ AUTHOR INFORMATION

Corresponding Authors

Olga Iranzo − *Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, 13397 Marseille, France;* orcid.org/0000-0001-8542-2429; Email: olga.iranzo@univ-amu.fr

Surajit Sinha − *School of Applied and Interdisciplinary Sciences, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India;* orcid.org/0000-0001-8884-1194; Email: ocss5@iacs.res.in

Authors

Isita Banerjee − *School of Applied and Interdisciplinary Sciences, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India*

Keshab Ch Ghosh − *School of Applied and Interdisciplinary Sciences, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India*

Emmanuel Oheix − *Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, 13397 Marseille, France*

Marion Jean − *Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, 13397 Marseille, France;* orcid.org/0000-0003-0524-8825

Jean-Valère Naubron − *Aix Marseille Univ, CNRS, Centrale Marseille, FSCM-Spectropole,, 13397 Marseille, France;* orcid.org/0000-0002-8523-4476

Marius Réglier − *Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, 13397 Marseille, France*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.0c02359

Notes

The authors declare no competing financial interest.

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