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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

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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

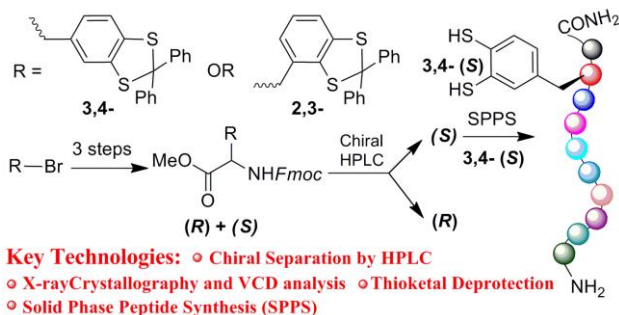
Isita Banerjee,[‡] Keshab Ch Ghosh,[‡] Emmanuel Oheix,[§] Marion Jean,[§] NAUBRON Jean-Valère,^{||} Marius Réglier,[§] Olga Iranzo,^{§,*} and Surajit Sinha^{‡,*}

[‡] School of Applied and Interdisciplinary Sciences, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700 032, India, *ocss5@iacs.res.in

[§] Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, 13397 Marseille, France,

*olga.iranzo@univ-amu.fr

^{||} Aix Marseille Univ, CNRS, Centrale Marseille, FSCM-Spectropole, 13397 Marseille, France



ABSTRACT

3,4- and 2,3-dimercaptophenylalanines have been synthesized for the first time by nucleophilic substitution of a protected aminomalonate on 3,4- and 2,3-dimercaptobenzylbromide derivatives. The dithiol functions were protected as thioketals and the key precursors, diphenylthioetal-protected dimercaptobenzylbromides, 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 H₂S 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 having 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 soft ligand having a high affinity for metals (Fe, Cu, Hg, Cd, Zn, Mo, W ...) are certainly the most significant.^{1,4} Additionally, cysteine or cysteine-containing peptides are useful in the generation of functionalized nanoparticles.^{4b,4d-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 non-canonical thiol-containing amino acids have been reported⁵ 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 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 a 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}

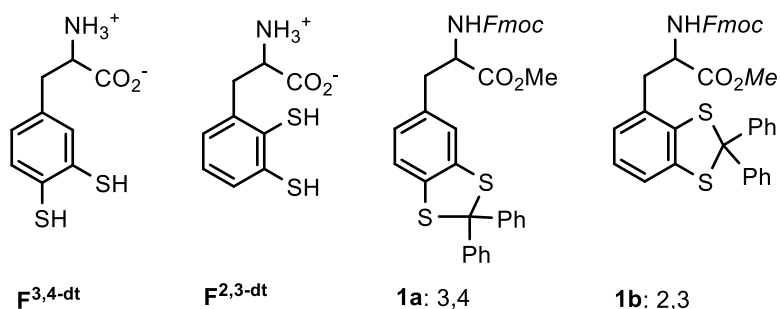


Figure 1. Structures of 3, 4- and 2, 3-dimercaptophenylalanine residues (**F^{3,4}-dt** and **F^{2,3}-dt**) and the fully protected amino-acids building blocks (**1a** and **1b**).

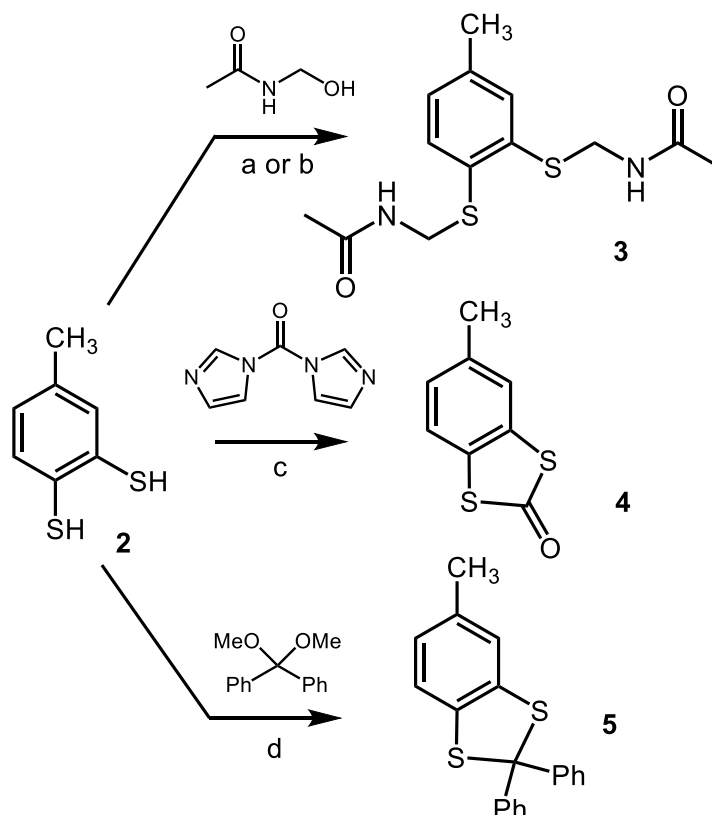
Indeed, to the best of our knowledge, no amino-acid bearing the dithiolene (alkene-1,2-dithiolate) function have been reported to date. These new building blocks can facilitate the introduction of dithiol groups and endow peptides/proteins with new structures and/or functionalities as previously mentioned.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

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 thiols⁸ or dithiols,^{6a} and they constitute promising candidates. Moreover, the dithiol function can be regenerated upon treatment with 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 for alternatives.

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



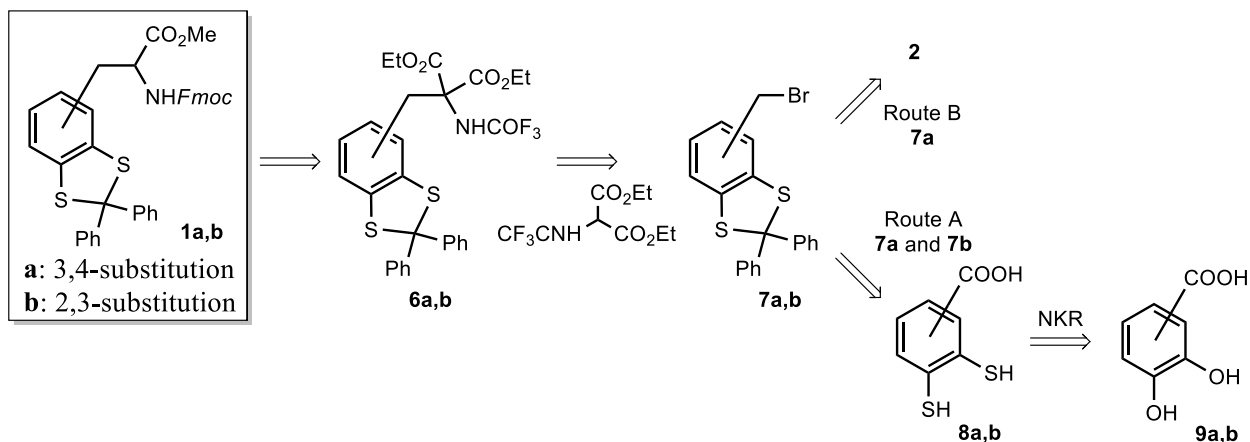
Scheme 1. Protection of aryldithiol: Reagents and conditions: Pathway a) *N*-(hydroxymethyl)acetamide (3.2 equiv), conc. HCl, MeOH, RT, 20 h: 45%; Pathway b) *N*-(hydroxymethyl)acetamide (3.2 equiv), TMSCl (2 equiv), THF, RT, 16 h: 48%; Pathway c) Carbonyldiimidazole (1.2 equiv), THF, RT, 18 h, 92%; Pathway d) $\text{Ph}_2(\text{OMe})_2$ (1.2 equiv), *p*-TSA (10 mol%), Toluene, 12 h, reflux, 98%.

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 1 h reaction and drying *in vacuo*, ¹H 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.

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 thioacetal or thioketal. Since we were interested on limiting the number of chiral centres 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 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-catalysed (*p*-toluenesulfonic acid) ketalization in dry toluene after 15 h reflux. Different conditions (BF₃.OEt₂, 53% and FeCl₃, 57%) (Table S1) were exploited but the yield has not been improved. In contrast, replacing benzophenone for its dimethyl ketal derivative¹³ 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.

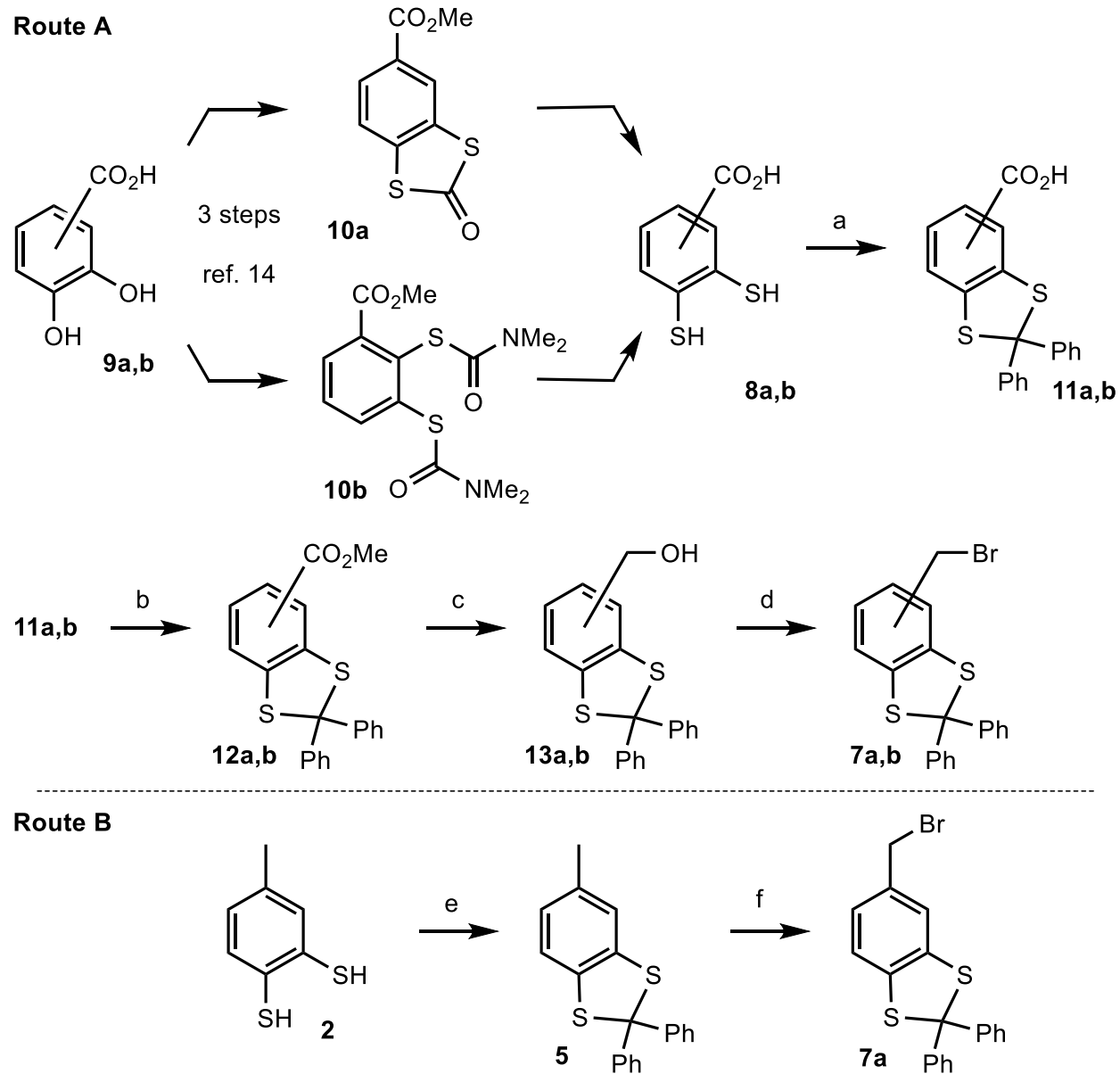
Synthesis of racemic mixtures 1a and 1b.

The specific aim of this work is to prepare **F**^{3,4-dt} and **F**^{2,3-dt} as suitable building blocks for *Fmoc*-peptide synthesis. Towards this goal, the target molecules, **1a** and **1b**, will include methyl esters protected carboxylates, *Fmoc*-protected amines, and dithiol side-chains protected as thioketals. Our initial approaches towards the asymmetric synthesis of **F**^{3,4-dt} from L-DOPA (Scheme S1) and **F**^{2,3-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 towards racemic compounds **1a** and **1b** is in Scheme 2. The coupling of *N*-protected aminodiethylmalonate with suitably dithiol-protected dimercaptobenzylbromide shall afford **6a** and **6b**, which upon hydrolysis and reprotection lead to **1a** and **1b**. The key precursors dimercaptobenzylbromides (**7a/7b**) could 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 temperature leads to *S*-thiocarbamates.¹⁴ Alternatively, 3,4-dimercaptobenzylbromide (**7a**) can be prepared directly from toluene-3,4-dithiol **2** (Scheme 2, route B).



Scheme 2. Retrosynthetic route towards racemic compounds **1a** and **1b**.

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 **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).



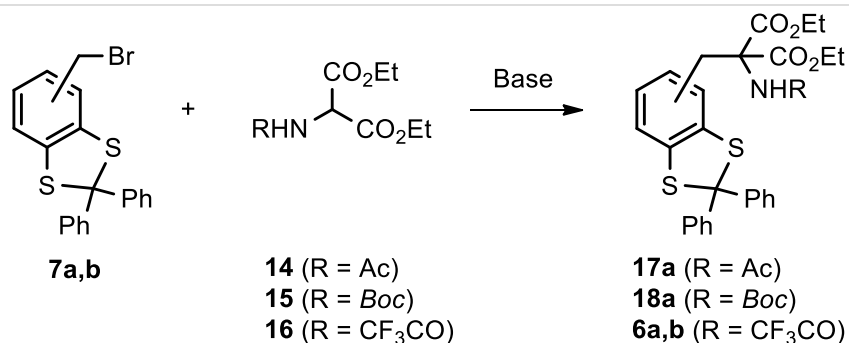
Scheme 3. Preparation of compounds **7a** and **7b**, reagents and conditions: a) $\text{Ph}_2(\text{OMe})_2$ (1.2 equiv), *p*-TSA (10 mol%), toluene, reflux, 12 h, **11a**: 84%, **11b**: 81% (from **10a/10b**); b) K_2CO_3 (2 equiv), CH_3I (4 equiv), DMF, 0°C to RT, 12 h, **12a**: 84%, **12b**: 81%; 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) $\text{Ph}_2(\text{OMe})_2$ (1.2 equiv), *p*-TSA (10 mol%), Toluene, 12 h, refluxed, **5**: 98%; f) NBS (1.1 equiv), CH_2Cl_2 , 1.5 h, refluxed, **7a**: 65%.

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 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** got decomposed in the reaction mixture. $\text{CBr}_4/\text{PPh}_3$ 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 Ac-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, 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 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 acetamide bond in the presence of NaOH in water:THF mixture. Hence, we used malonates **15** and **16**, where Boc group can be removed under 20% TFA in DCM and trifluoroacetamide can be removed in the presence of LiOH in 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 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 Cs₂CO₃ (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).

Table 1. Standardization of reaction conditions.

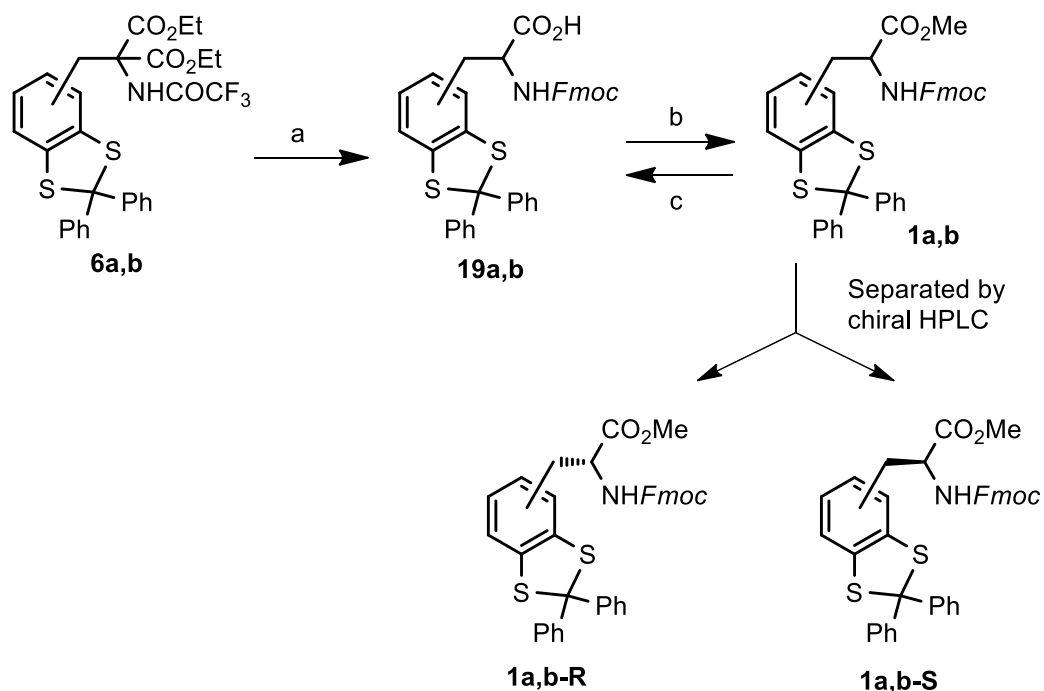


Entry	SM	Re	Base	S	T	t	Y
1	7a	14	NaH	DMF	RT	12 h	65
2	7a	15	NaH	DMF	RT	12 h	32

3	7a	15	NaH	DMF	70°C*	12 h	---
4	7a	15	NaOEt	EtOH	0°C	3 h	---
5	7a	15	Cs ₂ CO ₃	DMF	70°C*	12 h	---
6	7a	16	NaH	DMF	RT	12 h	40
7	7a	16	NaH	DMF	RT	6 h	82
8	7b	16	NaH	DMF	RT	6 h	75

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 inert atmosphere and allow 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 % (--- indicates that the reaction led to several unidentified product and/or starting material).

Then, the hydrolysis of ester function, decarboxylation reaction and hydrolysis of 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 K₂CO₃ 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 CaCl₂ and LiOH in a mixture of water, isopropanol and tetrahydrofuran.²⁰



Scheme 4. Synthesis of protected dimercaptophenylalanines **1a** and **1b**: Reagents and conditions: a) i) LiOH.H₂O (12 equiv), H₂O-THF (1:1), 70 °C, 12 h; ii) NaHCO₃, *Fmoc*-OSu (2 equiv), Dioxane-H₂O, RT, 12 h, **19a**: 75%, **19b**: 72%; b) CH₃I (4 equiv), K₂CO₃ (2 equiv), DMF, RT, 12 h, **1a**: 78%, **1b**: 77%; c) CaCl₂ (16 equiv), LiOH.H₂O (4 equiv), Isopropanol / THF/ H₂O (3:1:1.5), 2.5 h, **19a**: 98%, **19b**: 90%.

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 (Figures S3 for **1a**, S4 for **1b**), and optical rotation measurements (Tables S4 for **1a**, S5 for **1b**).

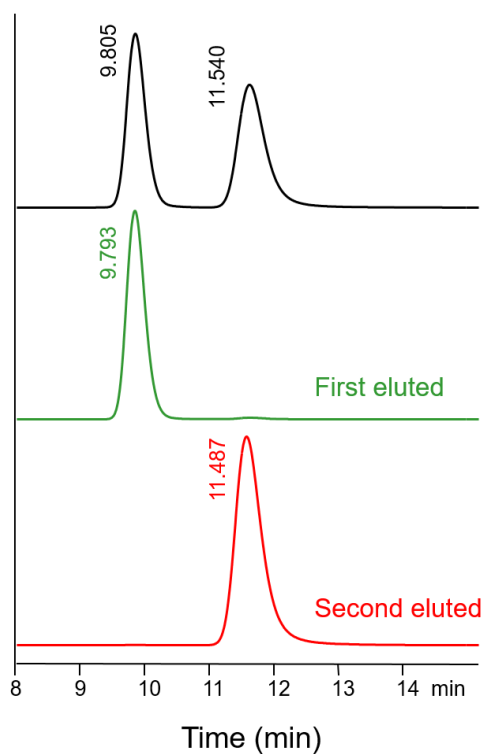


Figure 2. HPLC chromatogram (detection at 254 nm, 1 mL/min) recorded upon isocratic elution with heptane/isopropanol/dichloromethane (80/10/10) on analytical Chiralpak IF of (Top) racemic mixture of compound **1a**, (middle) purified enantiomer **1a-R**, (bottom) purified enantiomer **1a-S**.

In the case of **1a**, thin-needles of the enantiomer eluting first in HPLC were obtained and X-ray diffraction measurements allowed to determine the structure and the absolute configuration as (*R*) (Figures 3, S5, S6, Table S6). Hence, **1a-R** defines the compound eluting at 9.8 min in chiral HPLC (Figure 2). The absolute configuration assignment 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 CD₂Cl₂ and compared to the calculated averaged spectrum of the **1a-R** enantiomer (Figure 3). Calculations were performed using 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 Boltzmann population higher than 3% have been retained to build the averaged VCD spectrum (Figures S7–S9, Table S7).

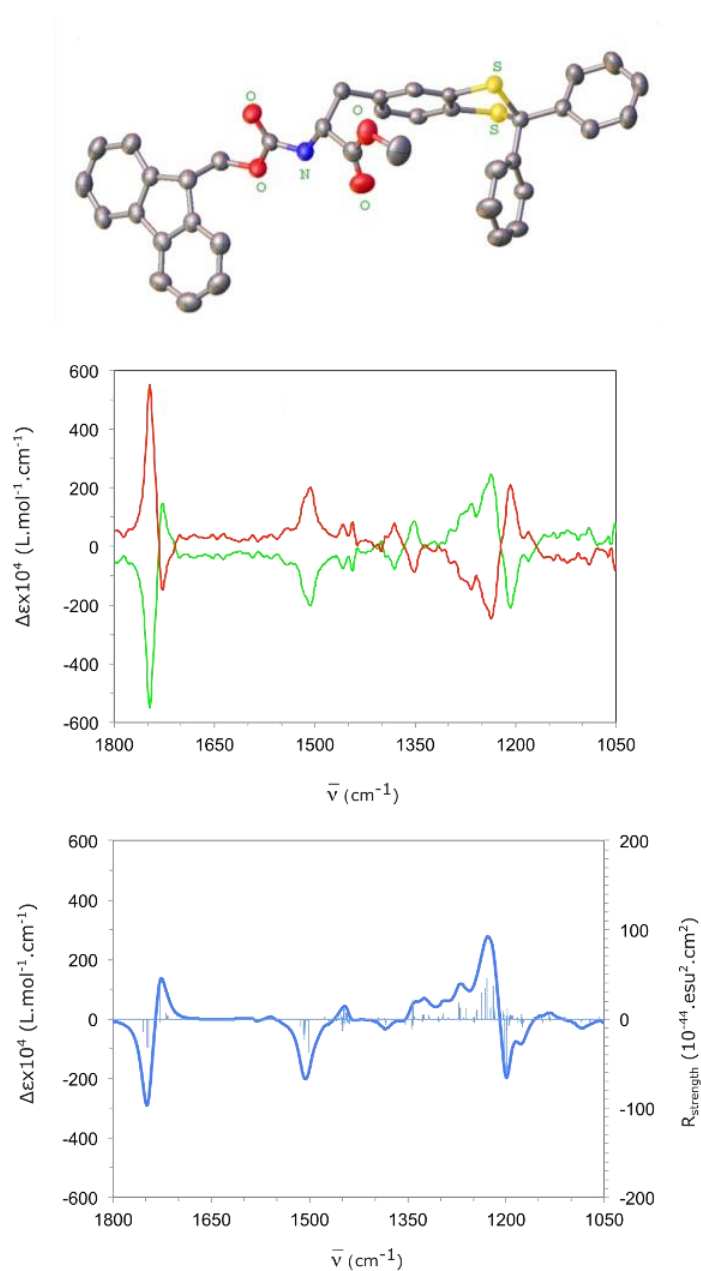


Figure 3. (Top) ORTEP representation of the X-ray structure of the first eluted enantiomer of compound **1a** with 50% probability thermal ellipsoids and partial atom labeling scheme (Hydrogen atoms are omitted for clarity). (Middle) Experimental VCD spectra recorded in CD₂Cl₂ at 298 K of (1st eluted)-**1a** (green) and (2nd eluted)-**1a** (red). (Bottom) Calculated averaged VCD spectrum for **1a-R** (dark blue).

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 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 unambiguously the absolute configuration (*R*) of the first eluted enantiomer of **1b** (Figures S10–S11, Table S8).

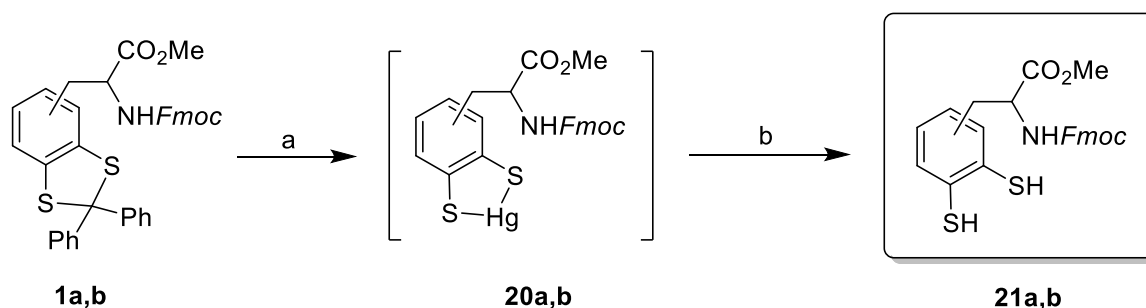
In order to use these building blocks in peptide synthesis, the acid function must be deprotected. For this, compound **1a-R** and **1a-S** were individually treated with CaCl₂ and LiOH solution²⁰ to afford **19a-R** and **19a-S** in 98% and 90% yields, respectively following the protocol of racemic mixtures reported in scheme 4. Analytical chiral HPLC confirmed that the deprotection conditions did not lead to racemisation 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 excess of H₂S. Following a reported procedure,²² deprotection of racemic amino acids **1a** and **1b** was attempted with HgCl₂ 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 AgNO₃,²² AgNO₂,²² or Na/naphthalene²³ did not give any

desired product. Treatment with triethylsilane in 50% TFA²⁴ 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 HBF₄ 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 HBF₄ and benzophenone, respectively gave rise to the Hg-complex **20** as a pale yellow precipitate. Next, H₂S gas was flown into a suspension of **20** in EtOAc under 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 Ar balloon. It is worth to mention here that isolation of dimercaptopropanol from Hg-complex by the decomposition of H₂S(g) was not possible earlier perhaps, presence of oxygen may have substantial effect in the isolation of free dithiol.²⁵ As X-ray photo electron 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 shown 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, S14). These values were in good agreement with the reported data of sulphur.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, Hg 4d level is preferred over 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 Inf.).

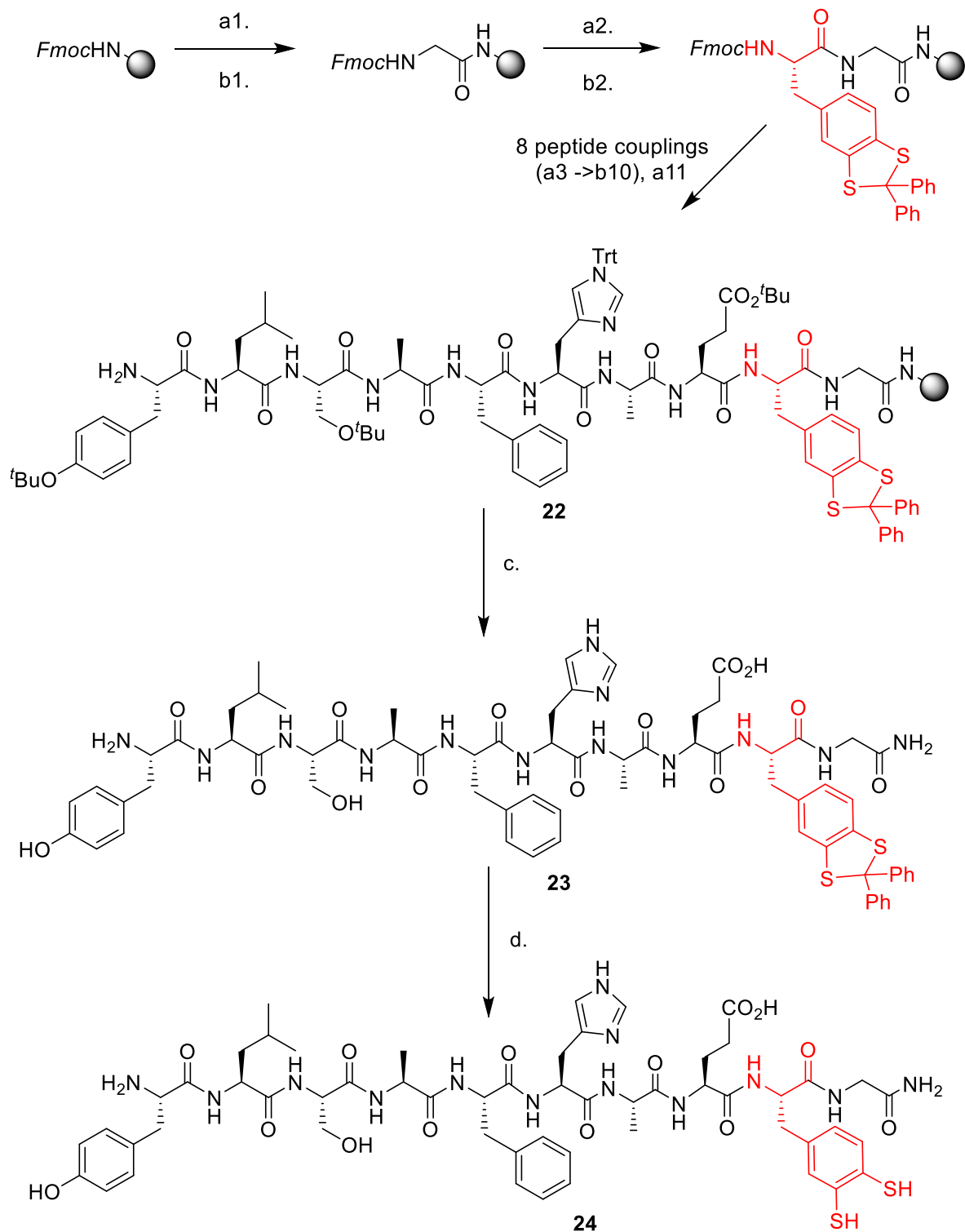


Scheme 5. Synthesis of dimercaptophenylalanines: Reagents and conditions: a) HgO (1.5 equiv), HBF₄ 35 wt. % in H₂O (6.8 equiv), THF, RT; b) H₂S (g), EtOAc, RT, 4h, Ar maintained by Schlenck line, **21a**: 44%, **21b**: 43%.

Solid-phase peptide synthesis of a decapeptide containing L-Phe^{3,4-dt} (= L-F^{3,4-dt})

In order to test the applicability of the noncanonical amino-acid L-Phe^{3,4-dt} in solid-phase peptide synthesis, we prepared the decapeptide H₂N-Tyr-Leu-Ser-Ala-Phe-His-Ala-Glu-Phe^{3,4-dt}-Gly-CONH₂ bearing only L-residues and different side chain functionalities. At first, sequence H₂N-Tyr(O^tBu)-Leu-Ser(O^tBu)-Ala-Phe-His(Trt)-Ala-Glu(O^tBu)-Phe^{3,4-dt}(CPh₂)-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 non-canonical amino-acid **19a-S** was incorporated by manual synthesis in the 2nd position starting from C-terminus in order to test its stability during 8 successive amino-acid couplings (Scheme 6). After final *Fmoc* deprotection, the 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 dialysis bag for 48 hours. The absence of Hg was confirmed by XPS analysis, displaying no peak for 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 SPOLAR C18 S5 column (Figure S16). In IR spectroscopy a peak at 2535 cm^{-1} was observed which strongly suggested the presence of free –SH group. In any case, the preparation of the 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 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 $[\text{M} + \text{Na}]^+$ and $[\text{M} + \text{K}]^+$, 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 $[\text{M} - \text{H} + \text{SH} + \text{Na}]^+$ and $[\text{M} - \text{H} + \text{SH} + \text{K}]^+$, respectively as expected based on the literature reports.²⁸ DTT-mediated persulfide reduction gave the desired product **24**.



Scheme 6. Synthesis of a decapeptide containing the dithiol function. Reagents and conditions:

a) 20% piperidine in DMF (2 x 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) first: **19a-S** (2 equiv), HBTU (2 equiv), DIEA (4 equiv), DMF, RT, 60 min; second: **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), HBF₄ 35 wt. % in H₂O (6.8 equiv), RT, THF, 2 h ; (ii) H₂S gas, EtOAc/MeOH (95:5), RT, 4 h.

CONCLUSION

We describe herein a convenient method for the preparation of racemic 3,4- and 2,3-dimercaptophenylalanines, as new entries in the non-canonical amino acid library. The key synthetic step involves the deprotection of thioketal function to obtain the 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-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.

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 60–80 °C. The following abbreviations are used for MeCN = acetonitrile, THF = tetrahydrofuran, DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, Et₂O = diethylether, *p*-TSA = *p*-Toluenesulfonic acid, TFA = trifluoroacetic acid, TIS = tris(isopropyl)silane. All reactions were carried out in oven-dried glassware under an argon atmosphere using anhydrous solvents, standard syringe and septum techniques unless otherwise indicated. Organic extracts were dried over anhydrous Na₂SO₄ 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. Electronic Circular Dichroism spectra 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). ¹H and ¹³C NMR spectra were recorded at 300 or 500 MHz and 75 or 125 MHz, respectively using CDCl₃ and CD₃OD as solvent. Chemical shifts (δ) are given in ppm relative to the solvent residual peak or TMS as 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 in a QTOF I (quadrupole-hexapole-TOF) mass spectrometer with an orthogonal Z-spray-electrospray interface on Micro (YA-263) mass spectrometer (Manchester, UK) and Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectra were recorded on Bruker ultrafleXtreme MALDI-TOF system. An automated peptide synthesiser Biotage Initiator + Alstra was used for the solid-phase peptide synthesis. All *Fmoc*-protected amino acids were purchased as L-enantiomers from Novabiochem.

Route a: *N,N'*-(4-methyl-1,2-phenylene)bis(sulfanediyl)bis-(methylene)diacetamide (3)

using HCl: To 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 for four times. The crude product was purified by silica gel column chromatography using DCM/MeOH (95:5) to obtain the product **3** (84 mg, 45%), ($R_f = 0.6$, DCM/MeOH, 95:5). ^1H NMR (400 MHz, CDCl_3): $\delta = 7.29$ (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. $^{13}\text{C}\{1\text{H}\}$ NMR (100 MHz, CDCl_3) $\delta = 170.5$, 138.8, 136.8, 133.6, 132.9, 131.9, 128.8, 43.4, 43.0, 23.0, 21.1 ppm. IR (Neat) $\nu_{\text{max}} = 3278$, 3064, 29.28, 1557, 1542, 1255 cm^{-1} . HRMS (ESI): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{13}\text{H}_{18}\text{O}_2\text{N}_2\text{S}_2\text{Na}$: 321.0707; found: 321.0706. MALDI-TOF: ($\text{M} = \text{C}_{13}\text{H}_{19}\text{N}_2\text{O}_2\text{S}_2$) 284.56 $[\text{M} - \text{CH}_3 + \text{H}]^+$.

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 3 times and the organic layers were combined, dried over sodium sulfate. The crude product was purified by silica gel column chromatography using DCM/MeOH (95:5) to obtain the 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 (SiO₂ / pentane: Et₂O 50:1). After 14 hours, 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 Na₂SO₄, 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). ¹H NMR (300 MHz, CDCl₃): 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. ¹³C{¹H} NMR (100 MHz, CDCl₃) 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 C₈H₇OS₂: 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), *p*-TSA (36.5 mg, 0.192 mmol) were dissolved in dry toluene and the reaction mixture was refluxed for 12 h in Dean-Stark apparatus. Then toluene was removed by evaporation and crude mixture was dissolved in chloroform and washed with 1N HCl, water and brine, dried over Na₂SO₄. The crude mixture was purified by flash column chromatography using Et₂O/Pet-ether (1:99) to give **5** as white solid (603 mg, 98% yield), (*R*_f = 0.6, Pet-ether/EtOAc, 95:5). ¹H NMR (300 MHz, CDCl₃): 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. ¹³C{¹H} NMR (75 MHz, CDCl₃): 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) *v*_{max} = 3055, 2918, 1489, 1457, 1443 cm⁻¹. HRMS (ESI): *m/z* [M]⁺ calcd for C₂₀H₁₆S₂: 320.0693; found: 320.0695.

Literature Preparations:

Compounds **9a₁**, **9b₁**, **9a₂**, **9b₂**, **10a**, **10b** were prepared according to reported literature procedures.¹⁵ Spectroscopic data of all 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 silicone oil bath under 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 Na₂SO₄ and concentrated to afford the product **8a**, as a yellow solid (361 mg, 92%). ¹H NMR (300 MHz, CD₃OD) δ = 7.97 (s, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.41 (d, *J* = 8.1

Hz, 1H) ppm. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CD_3OD) $\delta = 168.9, 140.4, 132.9, 131.4, 130.4, 129.4, 128.4$ ppm. IR (Neat) $\nu_{\text{max}} = 2924, 2854, 1681, 1586, 1314, 762$ cm^{-1} .

2,3-Dimercaptobenzoic acid (8b)^{15a}: Starting from the 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%). ^1H NMR (300 MHz, CD_3OD) $\delta = 7.86$ (d, $J = 7.8$ Hz, 1H), 7.54 (d, $J = 7.5$ Hz, 1H), 7.02 (t, $J = 7.8$ Hz, 1H) ppm. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CD_3OD) $\delta = 170.7, 138.5, 135.4, 133.5, 130.6, 128.7, 124.9$. IR (Neat) $\nu_{\text{max}} = 2497, 1664, 1395, 1314, 1268, 744$ cm^{-1} .

2,2-Diphenylbenzo[d][1,3]dithiole-5-carboxylic acid (11a): Compound **8a** together with dimethoxydiphenylmethane (474 mg, 2.081 mmol), pTSA (30 mg, 0.173 mmol) were dissolved in 5 mL dry toluene and the reaction mixture was refluxed in silicone oil bath for 12 h using Dean-Stark apparatus. Toluene was then removed by evaporation and crude mixture was dissolved in chloroform and washed with 1N HCl, water and brine, 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). ^1H NMR (300 MHz, CDCl_3): $\delta = 7.85$ (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. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 167.9, 143.8, 142.6, 138.3, 128.4, 128.1, 128.0, 127.9, 127.7, 123.1, 121.6, 78.3$ ppm. IR (Neat) $\nu_{\text{max}} = 3059, 2928, 1719, 1689, 1657, 1276$ cm^{-1} . HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{15}\text{O}_2\text{S}_2$: 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): $\delta = 7.75$ (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. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 168.2, 143.5, 141.9, 139.5, 128.2, 128.1, 127.6, 125.6, 125.3, 124.9, 75.5$ ppm. IR (Neat) $\nu_{\text{max}} = 3061, 2662, 1678, 1433, 1278, 750$ cm^{-1} . HRMS (ESI): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{14}\text{O}_2\text{S}_2\text{Na}$: 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), K_2CO_3 (209 mg, 1.51 mmol) was added 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), 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): $\delta = 7.53$ (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. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 166.3, 144.0, 142.7, 138.6, 128.3, 128.2, 128.1, 127.6, 123.0, 121.8, 78.5, 52.2$ ppm. IR (Neat) $\nu_{\text{max}} = 2950, 1717, 1582, 1430, 1285, 1234$ cm^{-1} . HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{21}\text{H}_{17}\text{O}_2\text{S}_2$: 365.0670; found: 365.0665.

Methyl 2,2-diphenylbenzo[d][1,3]dithiole-4-carboxylate (12b): **12b** was synthesized from compound **11b** (150 mg, 0.428 mmol) following the procedure of **12a**. 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): $\delta = 7.33$ (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. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 166.4, 143.5, 141.8, 139.7,$

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 $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{21}\text{H}_{16}\text{O}_2\text{S}_2\text{Na}$: 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 argon atmosphere at 0 °C. The reaction mixture was stirred at the same temperature for 5 min, 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. $^{13}\text{C}\{^1\text{H}\}$ 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 $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{16}\text{OS}_2\text{Na}$: 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**. 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, CDCl₃): 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. ¹³C{¹H} NMR (100 MHz, CDCl₃): 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 C₂₀H₁₆OS₂Na: 359.0540; found: 359.0541.

5-(Bromomethyl)-2, 2-diphenylbenzo[d][1,3]dithiol (7a):

Route A: To an ice-cold solution of PBr₃ (0.055 ml, 0.588 mmol) in 4 ml of anhydrous Et₂O under argon was added dropwise a solution of compound **13a** (153 mg, 0.59 mmol) in 6 ml anhydrous Et₂O 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 Na₂SO₄ 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 CH₂Cl₂ (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) in silicone oil bath 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 x 150 mL of a 0.1 M NaHCO₃ solution, and then with 1 x 150 mL water. Drying over Na₂SO₄ 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): $\delta = 7.58 - 7.54$ (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. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 143.0, 138.8, 138.5, 135.9, 128.3, 128.0, 127.0, 122.8, 122.3, 78.4, 33.1$ ppm. IR (Neat) $\nu_{\text{max}} = 2960, 2922, 1633, 1423, 889, 690$ cm^{-1} . HRMS (ESI):³⁰ m/z [$\text{M} + \text{Na}$]⁺ calcd for $\text{C}_{20}\text{H}_{15}\text{BrS}_2\text{Na}$: 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 the **route A**. The 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): $\delta = 7.68 - 7.57$ (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. $^{13}\text{C}\{1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 155.4, 142.8, 142.7, 139.5, 138.6, 132.0, 131.2, 128.6, 128.3, 128.0, 127.3, 127.2, 127.0, 126.5, 126.4, 123.5, 122.5, 110.9, 78.2, 33.2, 32.6$ ppm. IR (Neat) $\nu_{\text{max}} = 3058, 1489, 1442, 1412, 753, 695$ cm^{-1} . HRMS (ESI): m/z ($[\text{M}-\text{Br} + \text{Na}]^+ 100\%$) calcd for $\text{C}_{20}\text{H}_{15}\text{S}_2\text{Na}$: 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 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 cooled 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 Na₂SO₄ 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, CDCl₃): 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. ¹³C{¹H} NMR (100 MHz, CDCl₃): 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, 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 C₂₉H₂₆F₃NO₅S₂K: 628.0842; found: 628.0844.

Diethyl 2-((2,2-diphenylbenzo[d][1,3]dithiol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido) malonate (6b): **6b** was synthesized from compound **7b** (189 mg, 0.473 mmol), following the same procedure of **6a**. **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, CDCl₃): 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. ¹³C{¹H} NMR (100 MHz, CDCl₃): 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. ν_{max} = 3386, 3055, 2984, 1729, 1519,

1444, 1216, 1167, 696 cm^{-1} . HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{29}\text{H}_{27}\text{F}_3\text{NO}_5\text{S}_2$: 590.1277; found: 590.1276.

Diethyl 2-acetamido-2-((2-phenylbenzo[d][1,3]dithiol-5-yl)methyl)malonate (17a): **17a** was synthesized from **7a** (170 mg, 0.425 mmol) and malonate derivative **14**, following the same procedure of **6a**. **17a** 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): $\delta = 7.53$ (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. $^{13}\text{C}\{^1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 169.2, 167.4, 143.1, 138.2, 136.9, 133.5, 128.2, 128.0, 127.6, 123.6, 122.1, 78.3, 67.2, 62.8, 37.5, 23.1, 14.1$ ppm. IR (Neat) $\nu_{\text{max}} = 3405, 3064, 2983, 1740, 1680, 1493, 1443, 1276, 1198, 696$ cm^{-1} . HRMS (ESI): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{29}\text{H}_{29}\text{NO}_5\text{S}_2\text{Na}$: 558.1385; found: 558.1387.

Diethyl 2-(tert-butoxycarbonylamino)-2-((2,2-diphenylbenzo[d][1,3]dithiol-5-yl)methyl)malonate (18a): **18a** was synthesized from **7a** (165 mg, 0.413 mmol) and malonate derivative **15**, following the same procedure of **6a**. Colorless oily product was obtained. Yield 32% (82 mg), ($R_f = 0.5$, Pet-ether/EtOAc, 80:20). ^1H NMR (300 MHz, CDCl_3): $\delta = 7.62$ (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. $^{13}\text{C}\{^1\text{H}\}$ NMR (75 MHz, CDCl_3): $\delta = 167.6, 154.0, 143.2, 138.1, 136.7, 133.6, 128.20, 128.18, 128.1, 127.9, 127.6, 123.9, 122.0, 80.4, 78.2, 67.2, 62.7, 38.0, 28.4, 14.1$ ppm. IR (Neat) $\nu_{\text{max}} = 3431, 2979, 1740, 1715, 1489$ cm^{-1} HRMS (ESI): m/z $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{32}\text{H}_{35}\text{NO}_6\text{S}_2\text{Na}$: 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:H₂O (1:1) (10 ml) was added LiOH.H₂O (120.38 mg, 5.016 mmol), the mixture was allowed to reflux for 12 h in 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 neutralised by adding solid NaHCO₃ 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, extracted with EtOAc (3 × 5 ml). The combined organic layers were washed with water, followed by brine, dried over Na₂SO₄ 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 esterification reaction without further purification. ¹H NMR (300 MHz, CDCl₃): 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. ¹³C{¹H} NMR (75 MHz, CDCl₃): 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) *v*_{max} = 3273, 1737, 1699, 1655, 1450, 891 cm⁻¹. HRMS (ESI): *m/z* [M + H]⁺ calcd for C₃₇H₃₀NO₄S₂: 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**. **19b** was obtained as a white foamy solid product. Yield 72%, (*R*_f = 0.5, DCM/MeOH, 92:8). ¹H

NMR (300 MHz, CDCl₃): 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. ¹³C{¹H} NMR (75 MHz, CDCl₃): 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 C₃₇H₂₉NO₄S₂Na: 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), K₂CO₃ (94 mg, 0.68 mmol) was added portion wise at 0 °C. The mixture was stirred at RT for 10 min and again cooled to 0 °C, treated with CH₃I (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 NH₄Cl (5 ml), extracted with EtOAc (3 × 5 ml). The organic layers were combined, washed with brine, dried over Na₂SO₄ 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, CDCl₃): 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. ¹³C{¹H} NMR (75 MHz, CDCl₃): 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 $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{38}\text{H}_{31}\text{NO}_4\text{S}_2\text{Na}$: 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**. **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. $^{13}\text{C}\{^1\text{H}\}$ 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 $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{38}\text{H}_{31}\text{NO}_4\text{S}_2\text{Na}$: 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, polarimeter and ECD and VCD.

Chromatographic conditions for 1a: Compound **1a** was pre-purified on a preparative Chiralpak ID to remove ~10% of unidentified impurities. Then 380 mg of the purified racemic were dissolved in 8 mL of a mixture CH_2Cl_2 /ethanol (1/1). It was injected on Chiralpak IF (250 \times 10 mm) in 160 \times 50 μL stacked injections (every 4.2 min), using hexane/iPrOH/DCM (80/10/10) as mobile phase with flow-rate 5 mL/min. and detected with an UV detector at 280 nm. 2 fractions were collected and analyzed on analytical Chiralpak IF using

heptane/iPrOH/DCM (80/10/10) as isocratic eluant: **1a-R**: 147 mg, Rt = 9.8 (ee > 99.5%), $[\alpha]_{\text{D}}^{25}$ (CHCl₃, c = 0.89) = -29. Crystals could be grown by slow evaporation at 4°C of a solution containing 110 mg of the first eluted enantiomer for **1a** in CH₂Cl₂/EtOH (1:1) in the presence of traces of pentane and water. The structure was deposited on the CCDC (CCDC 2035077). **1a-S**: 139 mg, Rt = 11.48 min (ee > 99.5%), $[\alpha]_{\text{D}}^{25}$ (CHCl₃, c = 0.90) = +29. Crystals could be grown in similar conditions than 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 × 10 mm) in 40 × 50 μL stacked injections (every 3.8 min), using hexane/iPrOH/DCM (70/10/20) as mobile phase with flow-rate 5 mL/min, and detected with an UV detector at 290 nm. 2 fractions were collected and analyzed on analytical Chiralpak ID using Heptane/iPrOH/CH₂Cl₂ (70/10/20) as isocratic eluant: (1) unidentified: 145 mg, Rt = 5.51 min (ee > 99%), $[\alpha]_{\text{D}}^{25}$ (CHCl₃, c = 1.16) = +10.5. (2) unidentified: 135 mg, Rt = 6.48 min (ee > 99.5%), $[\alpha]_{\text{D}}^{25}$ (CHCl₃, 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): The compound **1a** (84 mg, 0.133 mmol) was dissolved in isopropanol (2.5 ml) and THF (0.8 ml). CaCl₂ (237 g, 2.13 mmol) was added. Separately, LiOH.H₂O (22.4 mg, 0.533 mmol) was dissolved in H₂O (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 (K₂CO₃) (5 ml) as a cloudy white suspension. The aqueous layer was partitioned in Et₂O (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 Na₂SO₄, and concentrated to obtain **19a** as a white foamy solid in 98% yield (80.4 mg, 0.131 mmol). The ¹H NMR spectrum recorded on the product was matching 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 same as the synthesis of **19a**.

Methyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(3,4-dimercaptophenyl)propanoate (21a): HBF₄ 35 wt. % in H₂O (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 orange to colorless solution. After completion of the reaction (TLC monitoring), THF was evaporated to afford a sticky material. 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 H₂S (g) (**!!! Caution !!!** H₂S 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 2-3 times. Finally, the collected supernatants were combined and dried *in vacuo* to get a yellowish solid product which was directly submitted for characterization. Isolated yield of **21a** is 44% (36 mg). **¹H NMR** (400 MHz, CDCl₃) 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. **¹³C{¹H} NMR** (75 MHz, CDCl₃) 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 C₂₅H₂₃NO₄S₂Na = 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**. Isolated yield of **21b** was 43% (35 mg). **¹H NMR** (300 MHz, CDCl₃) 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. **¹³C{¹H} NMR** (75 MHz, CDCl₃) 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 C₂₅H₂₃NO₄S₂Na = 488.0966; found 488.0965.

Synthesis of H₂N-Tyr-Leu-Ser-Ala-Phe-His-Ala-Glu--Phe^{3,4-dt}(CPh₂)-Gly-CONH₂ (23) : The linear sequence *Fmoc*-Tyr(O^tBu)-Leu-Ser(O^tBu)-Ala-Phe-His(Trt)-Ala-Glu(O^tBu)-L-Phe^{3,4-dt}(CPh₂)-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 pre-swollen 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 micro-wave synthesizer at 75°C for 5 min after 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-dt}) was performed manually at 25°C for 60 min after addition of 2 equivalents of *Fmoc*-L-Phe^{3,4-dt}(CPh₂)-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 extra 1.1 equivalents of **19a-S** (same protocol as before). The following coupling steps were performed on the automated micro-wave 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 afterwards simultaneously cleaved from the resin and side-chain deprotected (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 DMF and 5 × 5 mL DCM. Then, the resin was treated with 5 mL of TFA/TIS/water (95/2.5/2.5) cocktail for 2 hours. The volume of solution was decreased by flushing N₂ at the top of the solution. The crude peptide was precipitated by the addition of cold Et₂O 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%). ¹H NMR (300 MHz, D₂O/CD₃OD (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 C₆₈H₈₃N₁₃O₁₄S₂: 684.7806; found: 684.7803.

Synthesis of H₂N-Tyr-Leu-Ser-Ala-Phe-His-Ala-Glu--Phe^{3,4-dt}-Gly-CONH₂ (24): The peptide **23** (10.4 mg, 7 μmol) dissolved in 1 mL dry THF was treated with HBF₄ 35 wt. % in H₂O (12 μL, 0.047 mmol) followed by 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 argon balloon and H₂S was flown for 15 min. The reaction mixture was stirred for an additional 4 h resulting into the formation of a black precipitate. The 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 peptide was then dialyzed in 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) R_T = 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 $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{55}\text{H}_{73}\text{N}_{13}\text{O}_{14}\text{S}_2\text{Na} = 1226.4739$; found 1230.220. $[\text{M} + \text{K}]^+$ calcd for $\text{C}_{55}\text{H}_{73}\text{N}_{13}\text{O}_{14}\text{S}_2\text{K} = 1242.4478$; found 1247.521. HRMS (ESI): m/z $[\text{M} + \text{H}]^+$ calcd for dithiete compound $\text{C}_{55}\text{H}_{72}\text{N}_{13}\text{O}_{14}\text{S}_2$ 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 1/4 retardation was used to modulate the handedness of the circular polarized light at 50 kHz. Demodulation was performed by a lock-in amplifier (SR830 DSP). An optical low-pass filter ($< 1800 \text{ cm}^{-1}$) before the photoelastic modulator was used to enhance the signal/noise ratio. A transmission cell equipped with BaF_2 windows and of 200 μm of optical pathlength 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 were corrected using the standard procedure of the half-substraction of the spectra of each enantiomer. For each individual spectrum, about 12000 scans were averaged at 4 cm^{-1} resolution (corresponding to 3 hours 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 semi-empirical level PM3, starting from a geometry optimized using density functional theory (DFT) with B3LYP functional and 6-311G(d,p) basis set and including solvent (CD₂Cl₂) effects with a polarizable continuum model (SMD). During the annealing, only the dihedral angles have been relaxed, the bonds lengths and the valences 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 SMD (CD₂Cl₂)/B3LYP/6-311G (d,p) level. Using the geometries that have converged, we calculated Boltzmann population in CD₂Cl₂ 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(CH₂Cl₂)/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 Lorentzian band shape with a half-width at half maximum of 8 cm⁻¹. All calculations were performed using Ampac10³¹ (simulated annealing) and 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 *K* α monochromatic source with an energy resolution of 0.5 eV.

The samples were prepared in 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.

Supporting Information Available: 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 are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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