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Synthesis of β-(*S*-methyl)thioaspartic acid and derivatives

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Abstract—β-(*S*-Methyl)thioaspartic acid occurs as a posttranslational modification at position 88 in *Escherichia coli* ribosomal protein S12, a position that is a mutational hotspot resulting in both antibiotic-resistant and antibiotic-sensitive phenotypes. Critical to research designed to determine the biological function of β-(*S*-methyl)thioaspartic acid will be the availability of synthetic β-(*S*-methyl)thioaspartic acid as well as derivatives designed for peptide incorporation. We report here the synthesis of β-(*S*-methyl)thioaspartic acid and derivatives. The installation of the β-methylthio moiety into the aspartic acid structure was accomplished by electrophilic sulfenylation of N-protected-L-aspartic acid derivatives with 2,4-dinitrophenyl methyl disulfide. Following this key transformation, we were able to prepare protected β-(*S*-methyl)thioaspartic acid derivative suitable for peptide coupling. Published by Elsevier Ltd.

1. Introduction

The ribosome is the universal macromolecular machine involved in translating mRNA transcript into polypeptides. Several antibiotics act by targeting protein biosynthesis, interacting with ribosomal structural proteins, rRNAs, and ribosomal-associated proteins.[1] These antibiotics are useful mechanistic tools since studies on antibiotic–ribosomal proteins interactions can be especially informative about the structure of the ribosome.[2] For example, mutations in ribosomal proteins L22, S5, and S12 have been observed to confer resistance to erythromycin,[3] spectinomycin,[4] and streptomycin,[5] respectively, in *Escherichia coli*. The role of ribosomal protein S12 in maintenance of translational accuracy has been well established.[6]

Genetic and biochemical analyses of ribosomes from streptomycin-resistant mutants implicated ribosomal protein S12 as the determinant of the various streptomycin phenotypes, including resistance, dependence, and pseudodependence. The aspartic acid in the *E. coli* ribosomal protein S12 is posttranslationally modified via a β-methylthiolation to form the β-(*S*-methyl)thioaspartic acid (**1**).[7] This modified residue is located at position D88, near the streptomycin binding site and in the midst of residues altered in streptomycin-resistant mutants. This modification has also been found to occur in the phototropic bacterium *Rhodopseudomonas palustris*,[8] and in the extremely thermophilic bacterium *Thermus thermophilus* in the same location.[9] Mutations in the immediate vicinity of residue 88 have functional consequences. For example, the mutation that confers streptomycin resistance corresponds to a single amino acid replacement of Lys → Arg at residue 87,[10] and these mutant ribosomes are capable of performing spontaneous nonenzymatic translocation in vitro.[11] However, recently it was reported that β-methylthiolation is not a determinant of the streptomycin phenotype in *T. thermophilus*.[12]

In bacteria, S12 binds to 16S rRNA in regions associated with the fidelity of codon recognition. When coupled with the parsimonious nature of bacterial genetics, it is likely that β-(*S*-methyl)thioaspartic acid is both structurally and functionally important. However, the synthesis of this amino acid was not reported yet and its role in ribosome function has not been delineated.

We report herein the synthesis of β-(*S*-methyl)thioaspartic acid (**1**) and selectively protected derivatives that are

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designed for incorporation into peptides. These peptides in turn will be used to elucidate the enzymology of post-transcriptional modification as part of a study to determine the biological function of this modification.

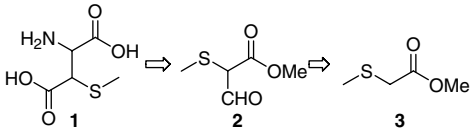
2. Results and discussion

For the synthesis of this amino acid **1**, we initially considered a Strecker synthesis shown retrosynthetically in Scheme 1. The aldehyde **2** was synthesized in 67% yield from methyl (methylthio)-acetate (**3**) by a published procedure.[13] However, the Strecker reaction of **2** with NaCN and NH_4Cl was unsatisfactory. Although, there was some ^1H NMR evidence of product formation, none was isolated. This poor result may be due to lowered reactivity of the aldehyde due to enolization as well as to isomerization of the intermediate imine to an enamine that is less reactive toward cyanide. The ^1H NMR shows that **2** is in equilibrium with the 3-hydroxyl-acrylates **2a** and **2b** in a ratio 7:53:40, respectively (Scheme 2).

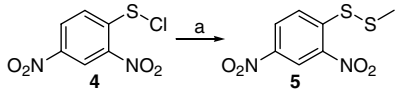
Attempts to increase the yield by functionalization of the aldehyde using less direct methods also failed. The reaction of **2** with *tert*-butyl carbamate[14] in the presence of $CuSO_4$ to form the *tert*-butylvinyl carbamate followed by in situ treatment with TMSCN produced only 28% of enamine and no α-aminonitrile was formed. Attempted 1,4 addition of TMSCN to the isolated enamine[15] under a variety of conditions also failed as did aminocyanation of **2** catalyzed by lithium perchlorate/diethylether (LPDE).[16]

Due to these failures to elaborate the amino acid side chain from **2**, we decided to install the methylthio moiety onto an appropriate β-amino ester enolate using electrophilic sulfenylation.[17] The necessary 2,4-dinitrophenyl methyl disulfide (**5**) was synthesized, on a multi-gram-scale, in 77% yield by microwave irradiation (130 °C) of a series of solutions of 2,4-dinitrobenzenesulfenyl chloride (**4**) in acetonitrile with 1 equiv of DMSO (Scheme 3).[18] The best result was obtained by irradiating several solutions of 500 mg of **4**, (0.43 M in acetonitrile) because irradiation on a gram-scale (2 g) resulted in lower yields (53%) of **5**.

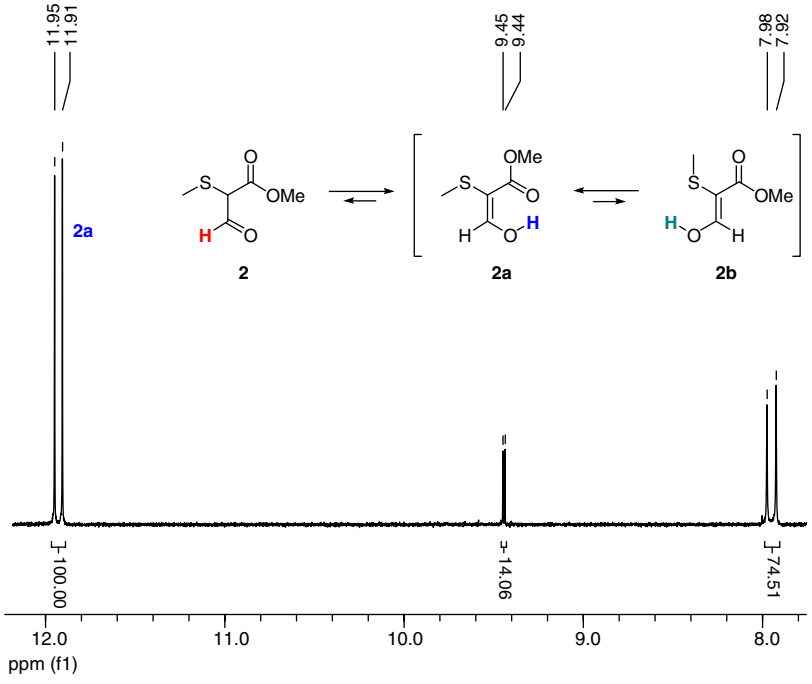
With **5** in hand, we next prepared substrates for electrophilic sulfenylation. The remaining carboxylic acid of Z-Asp(O*t*-Bu)OH **6a** was esterified with $TMSCHN_2$ in 15 min at room temperature[19] to afford **7a** in 87% yield. The reaction of **7a** with LDA, **5** and subsequent acidic hydrolysis of **8a** without isolation afforded the target amino acid **1**. After purification using ion exchange chromatography, 3-(*S*-methyl)thioaspartic acid **1** was obtained in 33% yield as a mixture (1:1) of both diaste-



Scheme 1. Retrosynthesis of amino acid **1**.



Scheme 3. Reagents and conditions: (a) DMSO 1 equiv, CH_3CN, MW, 130 ° C, 10 min.



Scheme 2. Portion of 1H NMR of **2** showing the equilibrium with the 3-hydroxyl-acrylates **2a** and **2b**.

reomers, contaminated with 17% of aspartic acid coming from unreactive **6a**. All attempts to isolate separate diastereomers were unsuccessful.

For biological studies involving peptide synthesis we required N-protected-3-methylsulfanyl-L-aspartic acid-4-esters. Because the selective hydrolysis of the methyl ester of **8a** while leaving the *tert*-butyl ester intact is problematic, we explored other protective groups more amenable to selective deprotection using the commercial available L-aspartic acid derivatives **6a, d**, and **e** as starting materials (Scheme 4).

Initially, we selected the TMDBS group as a good option for the protection of **6a**. Using TBDMSCl and imidazole the protected amino acid **7b** was obtained in 79% yield. However no evidence of **8b** was observed after the sulfenylation reaction.

The benzylation of **6a** with BnBr and Cs_2CO_3 using microwave irradiation at 130 °C for 10 min afforded **7c** in 93% yield. The sulfenylation of **7c** produced a complex mixture that complicated purification. However, we were able to isolate the product **8c** in 16% yield (Table 1). We have not investigated this transformation in detail and have no ready explanation for the low yields of methylsulfenylation product compared to higher reported yields of arylsulfenylation.[17] The electrophilic sulfenylation proceeded via an *anti* addition,[20] and it has been reported that the product obtained with aspartic acid derivative has the (2*R*,3*R*) configuration.[17a] Additionally, the configuration of the newly stereogenic center when (2,4-dimethoxybenzylthio)-4-methylphenyl sulfonate was used as sulfenylating agent has been confirmed by X-ray analysis.[17b] Comparisons of the H_α–H_β coupling constant of **8c** (4.9 Hz) and the products reported (4.5–5.4 Hz) suggested the same configuration. Unfortunately, deprotection of the benzyl ester under mildly basic conditions (0.1 N K_2CO_3, H_2O, and THF) failed. The benzyl group can be removed under harsher basic condition, but this would make retention of the *tert*-butyl ester difficult. Other conditions that would also remove the benzyl carbamate were not applicable. Due to the low yield obtained in the synthesis of **8c**, we abandoned this intermediate before exploring all options for deprotection and, instead, explored other protecting groups.

Table 1. Synthesis of N-protected-3-methylsulfanyl-L-aspartic acid derivatives

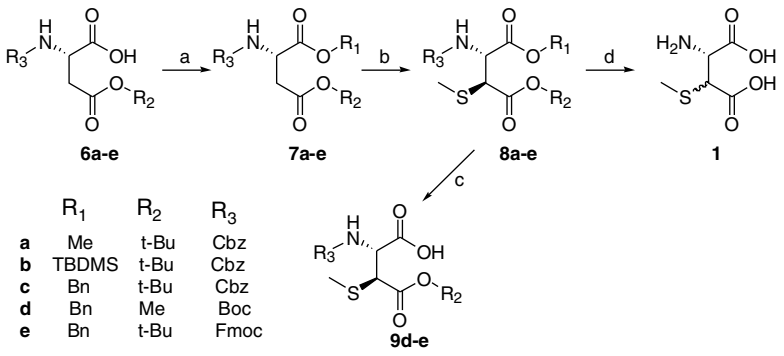
Compound	R_1	Yield **7** (%)	Base	Yield **8** (%)	Yield **9** (%)
7a	Me	87	—	—	—
7b	TBDMS	79	LDA	NR	—
7c	Bn	93	LDA	16	NR
7d	Bn	100	LDA	16	86
7e	Bn	85	LiHMDS	26	87

Another promising starting material for this reaction is the amino acid **6d**. To avoid thermolytic cleavage of the *tert*-butyl carbamate,[21] the benzylation was carried out at 60 °C with the cooling system in the microwave oven activated. Under those conditions, **7d** was obtained in almost quantitative yield. In the sulfenylation reaction, the monosulfenylated amino acid **8d** was isolated in 16% yield and 1% of disulfenylated product was observed by NMR. The benzyl ester was easily cleaved by transfer hydrogenation, using palladium-black with 1,4-cyclohexadiene as hydrogen donor.[22] The presence of the sulfur moiety did not poison the catalyst and the yield of **9d** was 86%.

We also explored the reaction using **6e** as a starting material. Using the same procedure, described above, the protected amino acid **7e** was obtained in 85% yield. Using LiHMDS as base, **8e** was obtained in 26% after the sulfenylation reaction. The cleavage under the same conditions affords the amino acid **9e** in 87% yield. This provides the Fmoc protected mono-*tert*-butyl ester of (2*R*,3*R*)-**1** as a single diastereomer, an orthogonally protected derivative suitable for peptide incorporation. The product appeared as a single peak in two HPLC systems (Chirobiotic R and C18 columns) supporting likely diastereomeric homogeneity.

3. Conclusion

The electrophilic sulfenylation of N-protected-L-aspartic acid-1-benzyl-4-esters (**7d**–**7e**) with 2,4-dinitrophenyl



Scheme 4. Reagents and conditions: (a) compound **7a**: $TMSCHN_2$ 2.4 equiv, toluene/MeOH 3:1, rt, 15 min; compound **7b**: TBDMSCl 1.1 equiv, imidazole 2.2 equiv, DMF, 0 °C, 5 min, rt, 3 days; compound **7c**–**7e**: BnBr 1.2 equiv, Cs_2CO_3 1.2 equiv, CH_3CN, MW, 60 °C or 130 °C, 10 min. (b) LiHMDS or LDA 2.2 equiv, compound **5** 1.4 equiv, −78 °C. (c) 1,4-cyclohexadiene 10 equiv, black Pd (equal weight), 25 °C, 3 h; (d) compound **8a**: 6 N HCl, reflux, 5 h.

methyl disulfide (**5**) affords (2*R*,3*R*)-N-protected-3-methylsulfanyl-L-aspartic acid-1-benzyl-4-esters **8d** and **8e** in 16% and 26% yields, respectively, as a single diastereomer. The benzyl ester was cleaved by catalytic transfer hydrogenation to afford the N-protected-3-methylsulfanyl-L-aspartic acid-4-esters **9d** and **9e** in high yield. These protected derivatives of amino acid **1** are suitable for peptide incorporation.

4. Experimental

4.1. General

All solvents and reagents were from Aldrich and used without further purification. NMR spectra were run in $CDCl_3$ on a Varian Gemini 300 MHz or Mercury 300 MHz spectrometer. Chemical shifts are expressed in ppm with TMS as internal reference. Mass spectra were determined using a Hewlett–Packard 1100 MSD instrument. Reactions were monitored by TLC on silica gel using 10% methanol in dichloromethane as solvent and compounds visualized by a UV lamp. Flash chromatography was carried out in a Biotage SP4™ Purification System. The microwave assisted reactions were run in a Biotage Initiator™ microwave oven. Optical rotations were determined with a JASCO P-2000 polarimeter. The reported yields are for purified material and are not optimized.

4.2. Synthesis of 2,4-dinitrophenyl methyl disulfide (3)

To a solution of 500.0 mg of 2,4-dinitrobenzene-sulfenyl chloride in 5 mL of acetonitrile was added 1.15 mL of DMSO, and the mixture was irradiated at 130 °C for 10 min (absorption level = normal). The undissolved material was removed by filtration and the solution was concentrated under reduced pressure. The crude product was purified by column chromatography (KP-Sil column, 0–20% of EtOAc in hexane, UV 235–245 nm) to afford 389.7 mg of product. Yellow needles; 77% yield; mp 96–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.48 (s, 3H), 8.50 (d, J = 1.4 Hz, 2H), 9.13 (t, J = 1.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 21.77, 121.64, 127.46, 128.23, 144.91, 145.43, 145.91; HRMS (TOF AP+) *m/z* calcd for $C_7H_7N_2O_4S_2$ $(M+H)^+$: 246.9847; found: 246.9846.

4.3. General procedure for the benzylation of N-protected-L-aspartic acid 4 esters

To a solution of 6 mmol of N-protected-L-aspartic acid β-esters in 10 mL of CH_3CN under N_2 were added 7.2 mmol of anhydrous Cs_2CO_3 and 7.2 mmol of BnBr, and the mixture was irradiated 10 min at 130 °C (normal absorption and cooling OFF) or 60 °C (normal absorption and cooling ON). The solid was removed by filtration and the solvent removed under reduced pressure. The crude product was purified by column chromatography (KP-SIL column, 7–60% of EtOAc in hexane, UV 235–245 nm) to afford the pure product.

4.3.1. *N*-Benzyloxycarbonyl-L-aspartic acid-1-benzyl ester-4-*tert*-butyl ester (7c).

Oil; 93% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.38 (s, 9H), 2.75 (dd, J = 16.9 Hz, J = 4.5 Hz, 1H), 2.96 (dd, J = 17.0 Hz, J = 4.6 Hz, 1H), 4.63 (ddd, J = 8.6 Hz, J = 4.6 Hz, J = 4.5 Hz, 1H), 5.12 (s, 2H), 5.15 (d, J = 12.4 Hz, 1H), 5.22 (d, J = 12.4 Hz, 1H), 5.77 (d, J = 8.9 Hz, 1H), 7.40–7.29 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.88, 37.66, 50.56, 66.97, 67.30, 81.71, 127.98, 128.08, 128.12, 128.29, 128.43, 128.48, 135.21, 136.14, 155.93, 169.79, 170.70; HRMS (TOF ES+) *m/z* calcd for $C_{23}H_{27}NO_6Na$ $(M+Na)^+$: 436.1736; found: 436.1729.

4.3.2. *N-tert*-Butoxycarbonyl-L-aspartic acid-1-benzyl ester-4-methyl ester (7d).

White needles; quantitative; mp 62–63 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.43 (s, 9H), 2.82 (dd, J = 17.0 Hz, J = 4.7 Hz, 1H), 3.02 (dd, J = 17.0 Hz, J = 4.5 Hz, 1H), 3.62 (s, 3H), 4.62 (ddd, J = 8.4 Hz, J = 4.7 Hz, J = 4.5 Hz, 1H), 5.15 (d, J = 12.3 Hz, 1H), 5.21 (d, J = 12.3 Hz, 1H), 5.50 (d, J = 8.4 Hz, 1H), 7.30–7.37 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 28.26, 36.64, 50.05, 51.93, 67.41, 80.14, 128.22, 128.37, 128.53, 135.26, 155.36, 170.88, 171.28; HRMS (TOF ES+) *m/z* calcd for $C_{17}H_{23}NO_6Na$: $(M+Na)^+$ 360.1423; found: 360.1427.

4.3.3. *N*-9*H*-Fluoren-9-ylmethoxycarbonyl-L-aspartic acid-1-benzyl ester-4-*tert*-butyl ester (7e).

White solid; 85% yield; mp 56–58 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.41 (s, 9H), 2.78 (dd, J = 16.9 Hz, J = 4.4 Hz, 1H), 2.97 (dd, J = 16.9 Hz, J = 4.7 Hz, 1H), 4.23 (dd, J = 7.3 Hz, J = 7.1 Hz, 1H), 4.33 (dd, J = 10.4 Hz, J = 7.3 Hz, 1H), 4.42 (dd, J = 10.4 Hz, J = 7.1 Hz, 1H), 4.65 (ddd, J = 8.7 Hz, J = 4.7 Hz, J = 4.4 Hz, 1H), 5.17 (d, J = 12.2 Hz, 1H), 5.24 (d, J = 12.2 Hz, 1H), 5.84 (d, J = 8.7 Hz, 1H), 7.44–7.26 (m, 9H), 7.59 (d, J = 7.6 Hz, 2H), 7.76 (d, J = 7.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.98, 37.72, 47.08, 50.63, 67.27, 67.45, 81.87, 119.95, 125.11, 125.16, 127.05, 127.69, 128.23, 128.40, 128.56, 135.23, 141.24, 141.27, 143.69, 143.89, 155.96, 169.93, 170.77; HRMS (TOF ES+) *m/z* calcd for $C_{30}H_{31}NO_6Na$: $(M+Na)^+$ 524.2049; found: 524.2043.

4.4. General procedure for the 3-sulfenylation of N-protected-L-aspartic acid-1-benzyl ester-4-esters

To a solution of 1 mmol of ester in 4 mL of anhydrous THF at −78 °C and under N_2 was added 2.2 mmol of LDA or LiHMDS over a period of 10 min. The solution was stirred for 30 min at this temperature and 1.2 mmol of 2,4-dinitrophenyl methyl disulfide was added in one portion as a solid. The mixture was stirred for 60 min at the same temperature and then allowed to reach −40 °C in 30 min. Then 3 mL of 2 N HCl was added and the product was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness in vacuum. The orange crude product was dissolved in hot cyclohexane and the undissolved material was removed by filtration. The solvent was removed under reduced pressure and the product purified by flash chromatography (KP-SIL column, 5–30% of EtOAc/CH_2Cl_2 (1:1) in cyclohexane, UV

235–245 nm). Recrystallization (EtOH/hexane) afforded the pure product.

4.4.1. (2*R*,3*R*)-*N*-Benzyloxycarbonyl-3-methylsulfanyl-aspartic acid-1-benzyl-ester-4-*tert*-butyl ester (8c). Oil; 16% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.40 (s, 9H), 2.25 (s, 3H), 3.78 (d, J = 4.9 Hz, 1H), 4.87 (dd, J = 10.1 Hz, J = 5.0 Hz, 1H), 5.24–5.08 (m, 4H), 5.92 (d, J = 10.1 Hz, 1H), 7.29–740 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 15.84, 27.83, 50.27, 55.28, 67.18, 67.53, 82.88, 127.95, 128.07, 128.23, 128.40, 128.45, 128.55, 135.06, 136.21, 156.48, 169.73, 169.89; HRMS (TOF ES+) *m*/*z* calcd for $C_{24}H_{30}NO_6S$: $(M+H)^+$ 460.1794; found: 460.1818.

4.4.2. (2*R*,3*R*)-*N*-*tert*-Butoxycarbonyl-3-methylsulfanyl-aspartic acid-1-benzyl ester-4-methyl ester (8d). Yellow needles; 16% yield; mp 83–85 °C; $[\alpha]_D^{27}$ +7.89° (*c* 0.76, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.44 (s, 9H), 2.26 (s, 3H), 3.64 (s, 3H), 3.87 (d, J = 4.8 Hz, 1H), 4.87 (dd, J = 10.1 Hz, J = 4.8 Hz, 1H), 5.11 (d, J = 12.2 Hz, 1H), 5.22 (d, J = 12.2 Hz, 1H), 5.61 (d, J = 10.1 Hz, 1H), 7.30–7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 15.88, 28.23, 49.68, 52.56, 54.75, 67.51, 80.27, 128.32, 128.43, 128.53, 135.08, 155.73, 169.86, 171.22; HRMS (TOF AP+) *m*/*z* calcd for $C_{18}H_{26}NO_6S$: $(M+H)^+$ 384.1481; found: 384.1466.

4.4.3. (2*R*,3*R*)-*N*-9*H*-Fluoren-9-ylmethoxycarbonyl-3-methylsulfanyl-aspartic acid-1-benzyl ester-4-*tert*-butyl ester (8e). White solid; 26% yield; mp 109.5–112.5 °C; $[\alpha]_D^{27}$ +0.95° (*c* 1.47, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.44 (s, 9H), 2.27 (s, 3H), 3.81 (d, J = 4.8 Hz, 1H), 4.25 (dd, J = 7.7 Hz, J = 7.0 Hz, 1H), 4.36 (dd, J = 10.4 Hz, J = 7.0 Hz, 1H), 4.42 (dd, J = 10.4 Hz, J = 7.7 Hz, 1H), 4.89 (dd, J = 10.2 Hz, J = 4.8 Hz, 1H), 5.19 (s, 2H), 5.99 (d, J = 10.2 Hz, 1H), 7.44–7.27 (m, 9H), 7.61 (d, J = 7.4 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 15.91, 27.89, 47.08, 50.30, 55.27, 67.52, 67.61, 82.97, 119.92, 125.23, 127.06, 127.65, 128.27, 128.45, 128.58, 135.04, 141.24, 143.79, 143.87, 156.48, 169.75, 170.05; HRMS (TOF ES+) *m*/*z* calcd for $C_{30}H_{33}NO_6$NaS: $(M+Na)^+$ 570.1926; found: 570.1916.

4.5. General procedure for the synthesis of N-protected-3-methylsulfanyl-L-aspartic acid-4-esters

To a well-stirred solution of 1.0 mmol of N-protected-3-methylsulfanyl-L-aspartic acid-1-benzyl ester-4-esters **8d–e** in 4 mL of absolute EtOH at 25 °C and under N_2 were added an equal weight of palladium-black and 10.0 mmol of 1,4-cyclohexadiene. The suspension was stirred at that temperature for 3 h. The catalyst was filtered over Celite and the solution was evaporated under reduced pressure to afford the product.

4.5.1. (2*R*,3*R*)-*N*-*tert*-Butoxycarbonyl-3-methylsulfanyl-aspartic acid-4-methyl ester (9d). Oil; 86% yield; $[\alpha]_D^{27}$ +22.66° (*c* 0.48, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 2.29 (s, 3H), 3.79 (s, 3H), 3.90 (d, J = 4.5 Hz, 1H), 4.83 (dd, J = 9.5 Hz, J = 4.4 Hz, 1H), 5.76 (d, J = 9.4 Hz, 1H), 7.80 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 16.05, 28.24, 49.21, 52.17, 52.89, 54.67, 80.76, 156.08, 171.96, 173.76; HRMS (TOF ES−) *m*/*z* calcd for $C_{11}H_{18}NO_6S$: (M−1) 292.0855; found: 292.0872.

4.5.2. (2*R*,3*R*)-*N*-9*H*-Fluoren-9-ylmethoxycarbonyl-3-methylsulfanyl-aspartic acid-4-*tert*-butyl ester (9e). Yellow solid; 87% yield; mp 55–57 °C; $[\alpha]_D^{27}$ +13.95° (*c* 0.52, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.50 (s, 9H), 2.28 (s, 3H), 3.81 (d, J = 4.4 Hz, 1H), 4.25 (dd, J = 7.7 Hz, J= 7.1 Hz, 1H), 4.34–4.45 (m, 2H), 4.84 (dd, J = 9.3 Hz, J = 4.4 Hz, 1H), 6.09 (d, J = 9.3 Hz, 1H), 7.29 (dt, J = 7.5 Hz, J = 0.6 Hz, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.61 (d, J = 7.3 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 16.08, 27.93, 47.04, 50.13, 55.15, 67.56, 83.58, 119.93, 125.18, 127.07, 127.68, 141.24, 143.72, 156.59, 171.10, 173.33; HRMS (TOF ES+) *m*/*z* calcd for $C_{24}H_{27}NO_6$NaS: $(M+Na)^+$ 480.1457; found: 480.1442.

Acknowledgments

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