Synthesis of both enantiomers of cyclic methionine analogue: (*R*)- and (*S*)-3-aminotetrahydrothiophene-3-carboxylic acids

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Key words: cyclic α,α-disubstituted α-amino acid; methionine analogue; optical resolution; chiral amino acid; unnatural amino acid

Abstract

A method of synthesizing an optically active cyclic methionine analogue, 3-aminotetrahydrothiophene-3-carboxylic acid (At_5c), is described. A Bucherer-Bergs reaction of 4,5-dihydro-3(2*H*)-thiophenone and the subsequent alkaline hydrolysis of hydantoin, followed by Cbz protection of the amine, afforded a racemic Cbz-At_5c (±)-**3** in excellent yield. Diastereomeric esters derived from Cbz-At_5c (±)-**3** and (*R*)-BINOL could be separated by column chromatography to give both diastereomers with >99% de. X-ray crystallographic analysis revealed the absolute configuration of synthesized amino acid derived from the less polar diastereomeric ester to be *S*.

1. Introduction

α,α-Disubstituted α-amino acids, non-proteinogenic amino acids possessing an additional α-alkyl substituent instead of hydrogen in α-amino acids, are promising tools for drug discovery [1], controlling peptide secondary structure [2], and protein engineering [3]. Much effort has been therefore devoted to designing and synthesizing optically active α,α-disubstituted α-amino acids [4]. 3-Aminotetrahydrothiophene-3-carboxylic acid (At_5c) is one of the methionine (Met) analogues expected to be biologically active (Fig. 1) [5]. Morimoto and Achiwa firstly reported the asymmetric synthesis of (—)-At_5c as a (—)-cucurbitine analogue [5a], which is known to inhibit the growth of immature *Schistosomas japonicum*. They used the enantioselective enzymatic hydrolysis of a prochiral ester, and synthesized (*S*)-(—)-At_5c. However, the enantiomeric excess of (*S*)-(—)-At_5c was only 6% ee. Guillerm *et al.* reported the kinetic resolution of a racemic Ac-At_5c-OMe using enzymatic hydrolysis for developing inhibitors of *S*-adenosyl L-methionine synthetase [5b]. They obtained one At_5c enantiomer with good enantiomeric selectivity (92% ee), but gave no description of the absolute configuration of the synthesized At_5c or the ee of the other enantiomer. Herein, we describe a concise method for producing highly enantiomeric (*S*)- and

(*R*)-At_5c. In addition, X-ray crystallographic analysis of the (+)-menthol derivative unambiguously revealed the absolute configuration of the synthesized At_5c.

2. Results and discussion

2.1. Synthesis of racemic amino acids.

According to a previous report [5b], the Bucherer-Bergs reaction of 4,5-dihydro-3(2*H*)-thiophenone using KCN and $(NH_4)_2CO_3$ gave a hydantoin **1** in 82% yield (Scheme 1). Hydrolysis of **1** with aqueous NaOH in a sealed tube, followed by Cbz protection of the amine using Cbz-OSu, gave a racemic cyclic amino acid, Cbz-At_5c (±)-**3,** in excellent yield (2 steps, >99%). Similarly, Boc protection of the amine in **2** was carried out using Boc_2O to give a racemic Boc-At_5c (±)-**4** in 97% yield.

2.2. Synthesis of optically active amino acids.

At first, we envisaged that optically active amino acids (*S*)- and (*R*)-At_5c could be prepared using optical resolution by recrystallization of diastereomeric salts between racemic carboxylic acids (amino acids) and chiral amines. The diastereomeric salts were prepared using (±)-**3** and (±)-**4** as racemic carboxylic acids and using (*S*)-phenylethylamine, (+)-cinchonine, and (—)-brucine as chiral amines, but only with the combination of (±)-**4** and (*S*)-phenylethylamine, were diastereomeric salts obtained as white crystals. Despite repeated recrystallization of the (±)-**4**/(*S*)-phenylethylamine salts, however, a complete optical resolution was not achieved.

Next, we thought that diastereomeric esters derived from racemic carboxylic acids and chiral alcohols could be separated by column chromatography. (*R*,*R*)-Cyclohexane-1,2-diol and (+)-menthol were used for preparing diastereomeric esters, which were diastereomeric mixtures with a ratio of 1 to 1 as determined from ^{1}H NMR spectra (data not shown). Unfortunately, the

diastereomers all showed similar spots on the TLC plate and could not be separated by column chromatography. Several attempts at optical resolution by recrystallization failed. Furthermore, (*R*)-BINOL was used as a chiral alcohol, but (±)-**4** (*R*)-BINOL esters were also inseparable. Finally, diastereomeric esters **5** prepared from (±)-**3** and (*R*)-BINOL (Scheme 1) showed two distinguishable spots on TLC. Column chromatography enabled them to be separated into less polar-**5** and more polar-**5** in 30% yields based on (±)-**3**, respectively. Note that diesters between (±)-**3** and (*R*)-BINOL were not detected in synthesizing the diastereomeric esters **5**. The ^{1}H NMR spectrum of the diastereomeric mixture **5** showed two doublet peaks of methylene protons in Cbz group at 5.12 and 5.16 ppm, and multiple peaks of C2 methylene protons in tetrahydrothiophene at 2.54—2.48 (2H), and 2.35 (1H) and 2.28 (1H) ppm, but those of less polar-**5** and more polar-**5** showed only one peak, indicating that the diastereomeric mixture could be absolutely separated by column chromatography (>99% de). Hydrolysis of less polar-**5** and more polar-**5** afforded (—)-**3** and (+)-**3**, respectively, which showed optical rotations $[\alpha]^{rt}_{D}$ = —3.39 and +3.13 with almost the same absolute values.

2.3. Determination of the absolute configuration of (—)-3

To determine the absolute configuration of the synthesized At_5c amino acid, (—)-**3** was coupled with (+)-menthol using EDC and DMAP in THF to afford the menthol ester **6** in 30% yield (Scheme 1). The menthol ester **6** formed crystals suitable for X-ray crystallographic analysis on the slow evaporation of *n*-hexane/EtOAc at room temperature (Table 1) [6]. The X-ray crystallographic analysis of **6** revealed the absolute configuration of (—)-**3** to be *S* (Fig. 2). The set of ϕ and ψ torsion angles of (*S*)-At_5c were —55.0˚ and —34.2˚, close to an ideal right-handed 3_{10}-helical structure (—57˚ and —30˚) [7].

3. Conclusion

We succeeded in developing a method of synthesizing optically active Met analogues At_5c with >99% ee starting from 4,5-dihydro-3(2*H*)-thiophenone. This method enabled us to obtain both enantiomers in five steps in 23% chemical yield. The X-ray crystallographic analysis of the (+)-menthol ester **6** revealed that the absolute configuration of (—)-**3** was *S*. The preparation of peptides having the optically active At_5c described here, and their conformational analysis are currently underway by our group.

4. Experimental Section

4.1. General.

Optical rotations $[\alpha]^{rt}_D$ were measured with a *Jasco DIP-370* polarimeter using a 0.5 or 1.0 dm cell. Infrared spectra (IR) were recorded on a *SHIMADZ IR Affinity-1* spectrometer for conventional measurements (KBr), and the solution ($CDCl_3$) method using a 0.1-mm path length of NaCl cell. ^{1}H NMR and ^{13}C NMR spectra were determined at *JEOL AL 400* (400 MHz) and *Varian UNITY plus 500* (500 MHz). FAB-MS spectra and DART-MS spectra were taken on *JEOL JMS-700N* and *JEOL JMS-T1000TD* spectrometers, respectively.

4.2. Synthesis and modification of amino acids.

*4.2.1. 7-Thia-1,3-diazaspiro[4.4]nonane-2,4-dione (**1**).*

KCN (16.0 g, 250 mmol) and $(NH_4)_2CO_3$ (53.7 g, 560 mmol) were added to a solution of 4,5-dihydro-3(2*H*)-thiophenone (22.8 g, 220 mmol) in ethanol (60 mL) / H_2O (80 mL) and stirred at 50˚C for 5 d. The resultant precipitate was isolated by filtration. A second crop was obtained from the filtrate by filtration of the resultant precipitate again. The combined resultant (63.2 g, 82%) was used for the next reaction without further purification: Colorless crystals; M.p. 248—250

˚C; IR (KBr) 3245, 3193, 3075, 2944, 2770, 1778, 1732 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.85 (br s, 1H), 8.46 (s, 1H), 3.07 (d, *J* = 11.2 Hz, 1H), 3.02—2.92 (m, 2H), 2.84 (d, *J* = 11.2 Hz, 1H), 2.16—2.02 (m, 2H); DART(+)HRMS calcd for $C_6H_9N_2O_2S$ (M^+ + H) 173.0385, found 173.0372.

*4.2.2. 3-(Benzyloxycarbonylamino)tetrahydrothiophene-3-carboxylic acid [(±)-**3**].*

1.1 M aqueous NaOH (33 mL, 36.3 mmol) was added to a stirred solution of **2** (1.53 g, 8.90 mmol) in MeOH (25 mL). After stirring at 150 ˚C in a sealed tube for 24 h, the solution was diluted with 1N aqueous HCl and evaporated in vacuo to leave a crude amino acid **2**. *N*-(Benzyloxycarbonyloxy)succinimide (Cbz-OSu, 3.59 g, 14.4 mmol) and Na_2CO_3 (1.24 g, 11.7 mmol) were added to a stirred solution of the crude amino acid in H_2O (50 mL) and THF (50 mL). After being stirred at room temperature for 5 d, THF was evaporated and the solution was washed with *n*-hexane. The solution was acidified with 1N aqueous HCl, extracted with EtOAc, and dried over $MgSO_4$. Removal of the solvent afforded crude (±)-**3** (2.51 g, >99%), which was used for the next reaction without further purification.

*4.2.3. 3-(tert-Butoxycarbonylamino)tetrahydrothiophene-3-carboxylic acid [(±)-**4**].*

1.1 M aqueous NaOH (28 mL, 30.2 mmol) was added to a stirred solution of **2** (1.13 g, 6.57 mmol) in MeOH (21 mL). After stirring at 150˚C in a sealed tube for 15 h, the solution was diluted with 3% aqueous HCl and evaporated in vacuo to leave a crude amino acid **2**. Di-*tert*-butyl dicarbonate (Boc_2O, 8.27 g, 37.9 mmol) and K_2CO_3 (5.24 g, 38.0 mmol) were added to a stirred solution of the crude amino acid in H_2O (8 mL) and acetone (12 mL). After being stirred at room temperature for 3 d, acetone was evaporated and the solution was washed with *n*-hexane. The solution was acidified with citric acid, extracted with $CHCl_3$, and dried over

$MgSO_4$. Removal of the solvent afforded crude (±)-**5** (1.57 g, 97%), which was used for the next reaction without further purification. Colorless crystals; IR (KBr) 3314, 2978, 2932, 1713, 1659 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.09 (br s, 1H), 3.39 (d, *J* = 11.7 Hz, 1H), 3.02 (d, *J* = 11.7 Hz, 1H), 2.98 (br s, 2H), 2.62 (br s, 1H), 2.39 (m, 1H), 1.45 (s, 9H); FAB(+)HRMS calcd for $C_{10}H_{18}NO_4S$ (M^+ + H) 248.0957, found 248.0934.

*4.2.4. 3-(Benzyloxycarbonylamino)tetrahydrothiophene-3-carboxylic acid (R)-2'-hydroxy-[1,1']-binaphthyl ester (**5**).*

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 2.29 g, 12.0 mmol), DMAP (1.32 g, 10.8 mmol), and (*R*)-BINOL (3.34 g, 11.6 mmol) were added to a stirred solution of (±)-**3** (2.51 g, 8.93 mmol) in THF (60 mL), and the solution was stirred at room temperature for 2 d. The solution was evaporated, diluted with $CHCl_3$, washed with 2% aqueous HCl, 5% aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel (400 g, 14% EtOAc in hexane) to give less polar-**5** (1.96 g, 30%) and more polar-**5** (1.96 g, 30%) as colorless crystals. Less polar-**5**: M.p. 86—88 ˚C; TLC (SiO_2, 25% EtOAc in hexane): R_f = 0.364; $[\alpha]^{26}_D$ = + 4.61 (c 1.06, MeOH); IR (KBr) 3387, 3059, 3034, 2947, 1759, 1717, 1508 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (m, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.53 (m, 1H), 7.39—7.29 (m, 10H), 7.23 (d, *J* = 8.2 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 5.12 (d, *J* = 9.6 Hz, 2H), 5.05 (br s, 1H), 5.04 (br s, 1H), 2.63—2.60 (m, 2H), 2.54—2.48 (m, 2H), 1.94 (m, 1H), 1.64 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 155.2, 151.7, 147.8, 135.9, 133.3, 133.2, 132.3, 130.8, 130.3, 128.8, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.4, 126.8, 126.3, 125.6, 124.5, 123.6, 122.6, 121.6, 118.1, 113.6, 68.1, 67.1, 38.4, 38.1, 28.4; FAB(+)HRMS calcd for $C_{33}H_{28}NO_5S$ (M^+ + H) 550.1688, found 550.1703. More polar-**5**: M.p. 80—82 ˚C; TLC (SiO_2, 25% EtOAc in

hexane): R_f = 0.303; $[\alpha]^{26}_D$ = + 12.9 (c 1.05, MeOH); IR (KBr) 3394, 3059, 3034, 2947, 1759, 1717, 1508 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (m, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.53 (m, 1H), 7.38—7.29 (m, 10H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.04 (d, *J* = 8.7 Hz, 1H), 5.16 (d, *J* = 12.1 Hz, 2H), 5.13 (br s, 1H), 5.08 (br s, 1H), 2.69—2.63 (m, 2H), 2.35 (m, 1H), 2.28 (m, 1H), 2.01—1.91 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 155.2, 151.7, 147.9, 133.3, 133.2, 132.4, 130.9, 130.4, 128.8, 128.61, 128.56, 128.4, 128.31, 128.28, 128.04, 127.97, 127.5, 126.9, 126.4, 125.6, 124.5, 123.7, 122.7, 121.6, 118.2, 113.7, 68.1, 67.1, 38.6, 37.5, 28.2; FAB(+)HRMS calcd for $C_{33}H_{28}NO_5S$ (M^+ + H) 550.1688, found 550.1707.

*4.2.5. (S)-3-(Benzyloxycalbonylamino)tetrahydrothiophene-3-carboxylic acid [Cbz-(S)-At_5c, (—)-**3**]*.

1.1 M aqueous NaOH (10 mL) was added to a solution of less polar-**5** (352 mg, 0.64 mmol) in MeOH (5 mL) at 0℃, and the solution was stirred at room temperature for 1 d. The solution was acidified with 1.1N aqueous HCl, and the MeOH was evaporated. The solution was extracted with EtOAC, and dried over Na_2SO_4. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel (EtOAc) to give (—)-**3** (171 mg, 95%) as colorless crystals. M.p. 148—150 ˚C; $[\alpha]^{26}_D$ = — 3.39 (c 1.00, MeOH); IR (KBr) 3391, 3375, 3067, 3038, 2980, 2945, 1744, 1670, 1533 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5H), 5.35 (br s, 1H), 5.13 (s, 2H), 3.37 (d, *J* = 12.0 Hz, 1H), 3.06—3.00 (m, 2H), 2.92 (m, 1H), 2.66 (m, 1H), 2.43 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 135.7, 128.6, 128.4, 128.1, 68.3, 67.4, 39.6, 38.4, 28.8; DART(+)HRMS calcd for $C_{13}H_{16}NO_4S$ (M^+ + H) 282.0800, found 282.0795. (+)-**3**: $[\alpha]^{29}_D$ = + 3.13 (c 0.99).

4.2.6. (3S)-(Benzyloxycarbonylamino)tertrahydrothiophene-3-carboxylic acid

*(1S,2R,5S)-2-isopropyl-5-methylcyclohexyl ester (**6**).*

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 55.7 mg, 0.29 mmol), DMAP (30.5 mg, 0.25 mmol), and (+)-menthol (54 mg, 0.35 mmol) were added to a stirred solution of (—)-**3** (52.6 mg, 0.19 mmol) in THF (1.5 mL), and the solution was stirred at room temperature for 1 d. The solution was evaporated, diluted with $CHCl_3$, washed with 2% aqueous HCl, 5% aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of the solvent afforded a residue, which was purified by column chromatography on silica gel (1% MeOH in chloroform) to give **6** (23.4 mg, 30%) as colorless crystals: M.p. 140—142 ˚C; $[\alpha]^{25}_D$ = + 7.18 (c 1.00, $CHCl_3$); IR (KBr) 3354, 2955, 1726, 1709 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5H), 5.23 (br s, 1H), 5.18—5.01 (ABX, J_{AX} = 12.2 Hz, J_{BX} = 12.2 Hz, J_{AB} = 54.5 Hz, 2H), 4.75—4.69 (dt, *J* = 3.9 Hz, *J* = 10.7 Hz, 1H), 3.28 (d, *J* = 11.5 Hz, 1H), 3.01—2.87 (m, 3H), 2.61 (m, 1H), 2.42 (m, 1H), 1.98 (m, 1H), 1.85 (m, 1H), 1.68—1.62 (m, 3H), 1.46—1.34 (m, 2H), 1.06 (m, 1H), 0.89—0.74 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 155.1, 128.5, 128.2, 128.0, 76.0, 68.6, 66.8, 46.9, 40.3, 39.6, 38.5, 34.1, 31.3, 28.8, 26.2, 23.2, 22.0, 20.8, 16.0; DART(+)HRMS calcd for $C_{23}H_{33}NO_4S$ (M^+ + H) 420.2209, found 420.2204.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (B) (2239022) from JSPS, by a Grant-in-Aid for Scientific Research on Innovative Area (24105525) from MEXT, by a Grant from the Naito Foundation, by a Grant from the Terumo Life Science Foundation, by a Grant from the Takeda Science Foundation, and by a Grant from the Mochida Memorial Foundation for Medical and Pharmaceutical Research.

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6. CCDC-919710 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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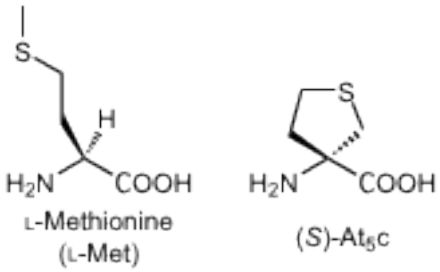


Figure 1. L-Methionine and its analogue (*S*)-At_5c.

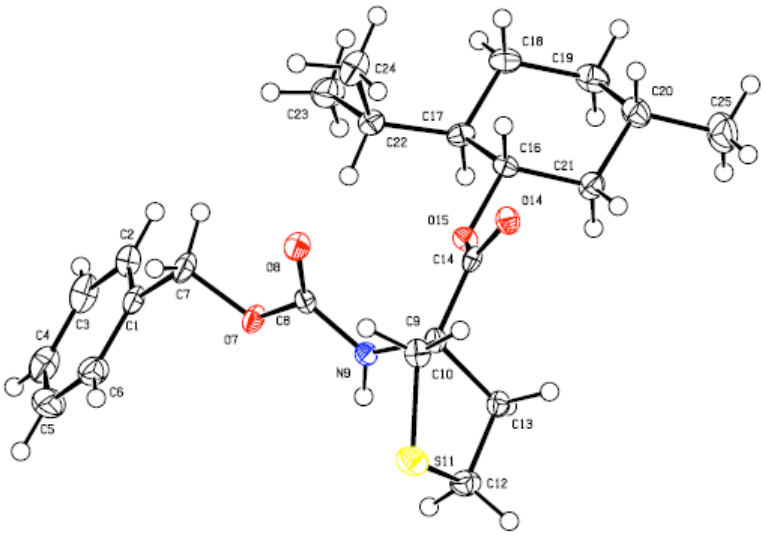
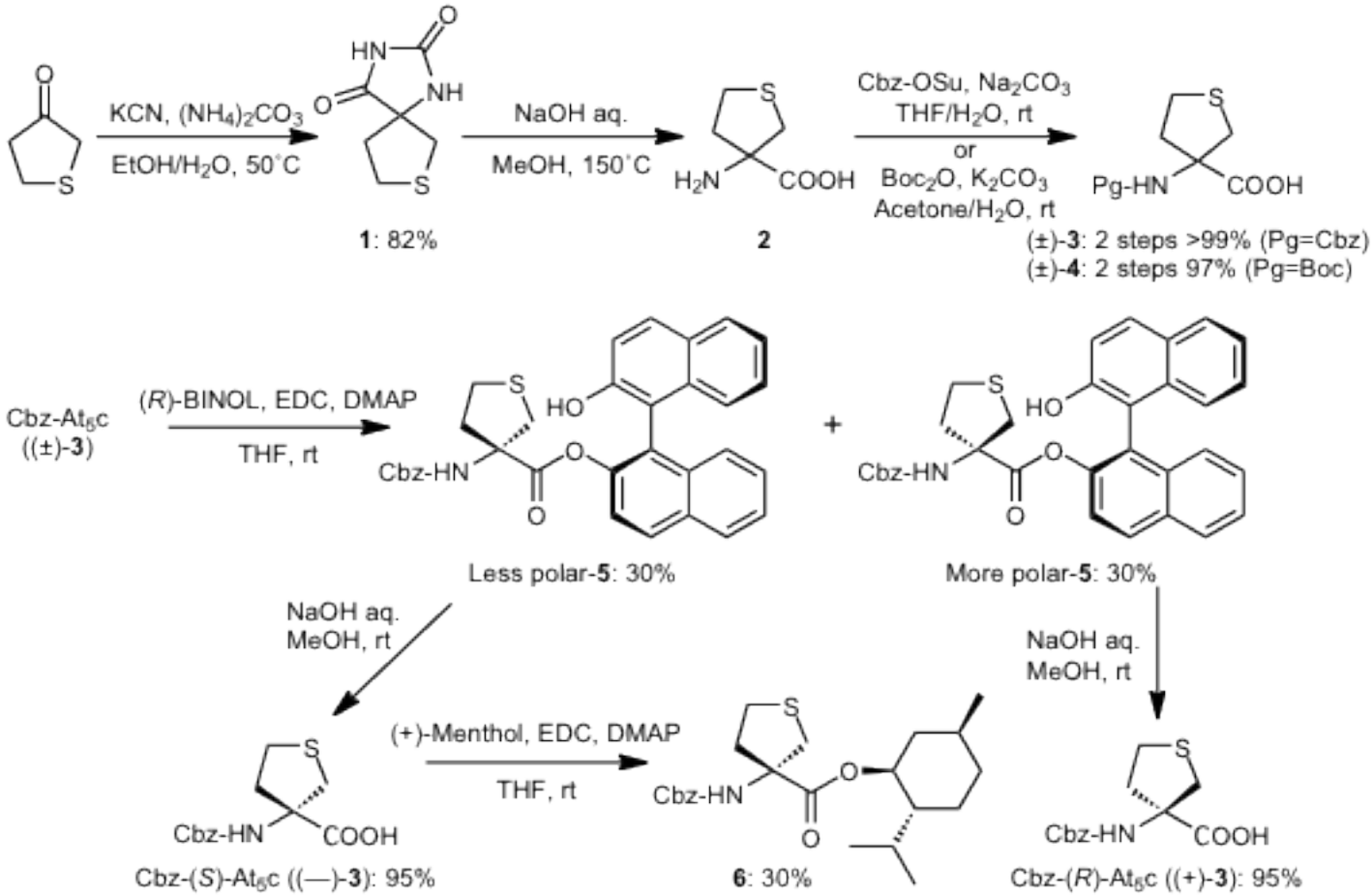


Figure 2. X-ray diffraction structure of **6**.



Scheme 1. Synthetic scheme of At_5c and its derivative.

Table 1. Crystal and diffraction parameters of **6**.

Empirical formula	$C_{23}H_{33}NO_4S$
Formula weight	419.56
Crystal dimensions (mm)	0.16×0.11×0.07
Crystal system	Orthorhombic
a, *b*, *c* (Å)	10.17, 11.79, 19.00
V (Å^3)	2277.8
Space group	$P2_12_12_1$
Z value	4
D_{calcd} (g/cm^3)	1.223
μ (MoKα) (cm^{-1})	0.170
No. of observation	4561 ($I>2\sigma(I)$)
No. of variables	262
R_1, R_w	0.0406, 0.0859
Solvent	*n*-hexane/EtOAc