Preparation of (+)-*Trans*-Isoalliin and Its Isomers by Chemical Synthesis and RP-HPLC Resolution

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Naturally occurring (+)-*trans*-isoalliin, (R_CR_S)-(+)-*trans*-S-1-propenyl-L-cysteine sulfoxide, is a major cysteine sulfoxide in onion. The importance of producing it synthetically to support further research is very well recognized. The (+)-*trans*-isoalliin is prepared by chemical synthesis and reversed-phase (RP)-HPLC. First, S-2-propenyl-L-cysteine (deoxyalliin) is formed from L-cysteine and allyl bromide, which is then isomerized to S-1-propenyl-L-cysteine (deoxyisoalliin) by a base-catalyzed reaction. A mixture of *cis* and *trans* forms of deoxyisoalliin is formed and separated by RP-HPLC. Oxidation of the *trans* form of deoxyisoalliin by H_2O_2 produces a mixture of (−)- and (+)-*trans*-isoalliin. Finally, RP-HPLC is used successfully in separating (−)- and (+)-*trans*-isoalliin, and hence, (+)-*trans*-isoalliin is synthesized for the first time in this study. In addition, the (±) diastereomers of *cis*-isoalliin are also separated and purified by RP-HPLC.

KEY WORDS: Allium cepa, 1-propenyl-L-cysteine sulfoxide, organosulfur compounds

INTRODUCTION

Naturally occurring (+)-*trans*-isoalliin, purified from onion (*Allium cepa*), (R_CR_S)-(+)-*trans*-S-1-propenyl-L-cysteine sulfoxide, is the precursor of onion's lachrymatory factor (propanethial S-oxide) and is involved in the formation of pigment precursors of pink discoloration of onion and green/blue discoloration of garlic (*Allium sativum*).[1–9] The (+)-*trans*-isoalliin can also serve as a standard for determination of cysteine sulfoxides in *Allium* plants, such as onion, leek, and garlic.[10] In addition, (+)-*trans*-isoalliin is an organosulfur compound from *Allium* plants with diverse health benefits.[2,11] To further the understanding of all of these subjects, an effective preparation of (+)-*trans*-isoalliin with adequate purity is needed, as it is not commercially available. There are reports of (+)-*trans*-isoalliin preparations by tedious and inefficient isolation from aqueous onion juice by means of ion exchange, recrystallization, and/or reversed-phase (RP)-HPLC (yield ~1 g from several kilograms of fresh onion bulbs), although none, so far, have been successful in chemically synthesizing (+)-*trans*-isoalliin.[1,8,12–14] In 1966 and 1983, two groups reported the synthesis of non-natural (±) diastereomers of *cis*-isoalliin,[15–17] whereas several research groups reported the synthesis of (±) diastereomers of *trans*-isoalliin.[18–21] Nishimura et al.[18] produced ethyl prop-2-ynyl sulfide, which was subsequently isomerized to ethyl prop-1-ynyl sulfide by mixing Na ethanethiolate with propargyl bromide. (±) Diastereomers of *trans*-isoalliin were produced through reductive coupling of ethyl prop-1-ynyl sulfide with propyl bromide by lithium in liquid NH_3 and oxidation of *trans*-prop-1-enyl-cysteine with H_2O_2. Parry et al.[19] reported the synthesis of (±) diastereomers of *trans*-isoalliin in five steps, using a similar reaction scheme without presenting experimental details after failed attempts of the method of Nishimura et al.[18] Recently, several papers have reported synthesis of (±)-*trans*-isoalliin using chemicals, such as palladium (Pd)-containing catalyst, *tert*-butyllithium/dibenzyl disulfide, Na metal, *tert*-butoxide, and H_2O_2.[20–23] In the first method, hydrochloride of L-cysteine ethyl ester is used as the starting material, and the final product is synthesized in five steps, including a special Pd-catalyzed coupling between carbon and sulfur as the key step.[20] In the second method, *trans*-1-bromo-1-propene is used as the starting material, and the final product is synthesized in three steps with the formation of vinyl sulfide in the first step, reductive cleavage/alkylation of vinyl sulfide in the second step, and oxidation of *trans*-S-1-propenyl-L-cystein (deoxyisoalliin) in the third step.[21] In the last method, deoxyisoalliin is formed by isomerization of S-2-propenyl-L-cysteine (deoxyalliin) with *tert*-butoxide and then oxidized by H_2O_2 to produce (±)-*trans*-isoalliin.22,23 However, in these meth-

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ods, a mixture of (+)-*trans*-isoalliin and (−)-*trans*-isoalliin is produced in the final synthesis.

Herein, we describe the facile preparation of (+)-*trans*-isoalliin by chemical synthesis and RP-HPLC purification. First, deoxyalliin is prepared by coupling L-cysteine and allyl bromide, which is purified directly from the reaction mixture by RP-HPLC. In the second step, deoxyallin is converted in a base-catalyzed isomerization to *cis* and *trans* forms of deoxyisoallin, which after separation by RP-HPLC, are converted in the third step to mixtures of (±) diastereomers of the respective *cis* and *trans* forms of isoalliin by oxidation with H_2O_2. The (±) diastereomers of *trans*-isoalliin are then separated and purified by RP-HPLC. In addition, the (±) diastereomers of *cis*-isoalliin are also separated and purified by RP-HPLC.

MATERIALS AND METHODS

Chemicals and General Methods

All reagents and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA), with the highest purity available and used without further purification. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker (Billerica, MA, USA) 900-MHz NMR spectrometer (900 MHz 1 H and 225 MHz 13C). NMR spectra were measured in D_2O at 25°C, and the chemical shifts were referenced to the trimethylsilyl propionate. Multiplicities are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; td, triplet of doublets; tt, triplet of triplets; q, quartet; dq, doublet of quartets; ddd, doublet of doubles of doubles; ddq, doublet of doubles of quartets. Electrospray ionization-high-resolution mass spectrometry (ESI-HRMS) spectra were recorded on a Q-TOF SYNAPT instrument (Waters, Milford, MA, USA) with a dual source and a suitable external calibrant. TLC was carried out on an aluminum-backed 0.25-mm silica gel plate with a fluorescence indicator (Dynamic Adsorbents, Norcross, GA, USA) and was developed with n-butanol:glacial acetic acid: water (2:1:1, v/v/v). Substance spots on the chromatographic plate were detected by illumination with UV light (254 nm), $KMnO_4$ staining, or 0.5% ninhydrin solution spraying. Optical rotation was measured with an Autopol IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA), using 40T polarimeter sample cells. Reaction products were purified using a Perseptive Biosystems (Life Technologies, Carlsbad, CA, USA) or an Agilent (Agilent Technologies, Santa Clara, CA, USA) HPLC system with a Phenomenex (Torrance, CA, USA) Kinetex RP-C18, 150 × 21.1-mm or 250 × 4.6-mm column. Gradient elution was run using Solvents A and B [Solvent A, 0.1% trifluoracetic acid(TFA) in water; Solvent B, 0.1% TFA in acetonitrile] from 100% A for 20 min with a linear increase to 100% B for another 5 min. The progress of the reaction was monitored by RP-HPLC. ^{1}H NMR spectrum in 10% D_2O of the resulting clear solution confirmed completion of the reaction. The products were confirmed by TLC and spectroscopic (^{1}H,^{13}C NMR, and ESI-HRMS) analysis. Yields refer to chromatographic and spectroscopic pure compounds.

Synthesis of Deoxyalliin (3)

The synthesis of Compound **3** was based on the procedure of Iberl et al.,[24] with modifications and with rigid temperature control throughout the reaction. L-Cysteine hydrochloride monohydrate (2 g, 0.01 mol) was dissolved in 2 mL distilled water and stirred at 0°C. A solution of Na hydroxide (1.6 g, 0.04 mol) in 2 mL distilled water was added drop-wise to the stirred reaction mixture with the temperature maintained at 0°C, followed by slowly adding allyl bromide (1 mL, 0.01 mol). The reaction mixture was then stirred for 14 h at 20°C. After completion, the reaction mixture was filtered and injected into a RP-HPLC to purify Compound **3**. The product fraction was dried as a white powder (1.2 g, 76% yield).

Syntheses of *Cis*-Deoxyisoalliin (4) and *Trans*-Deoxyisoalliin (5)

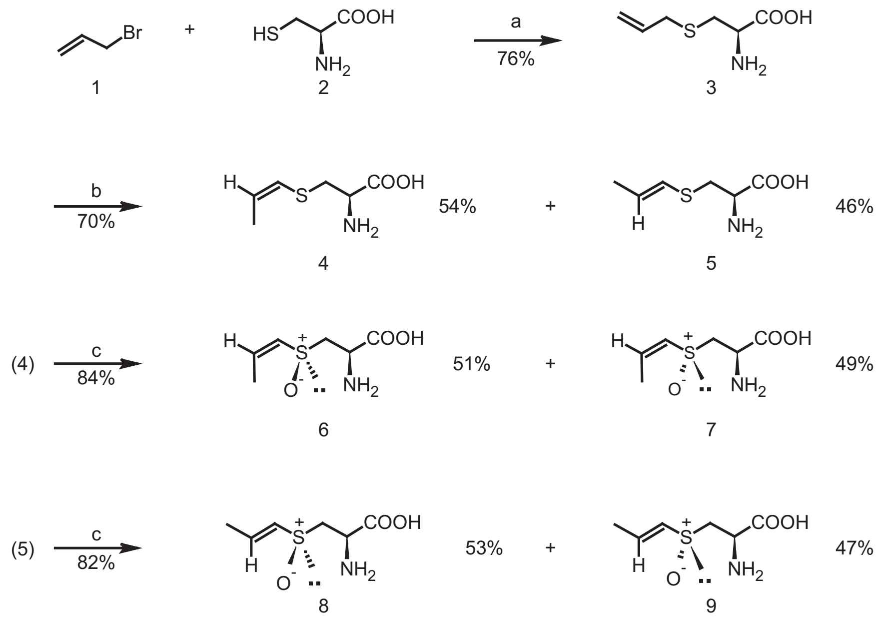
Deoxyisoalliins (**4** and **5**) were synthesized by base-catalyzed isomerization of deoxyalliin (**3**) with potassium *tert*-butoxide (KO*t*-Bu), according to the procedure of Carson et al.,[12] with some modifications. KO*t*-Bu (1.2 g, 10.6 mmol) was added with external cooling to a suspension of Compound **3** (1.2 g, 7.4 mmol) in 80 mL DMSO. The pale, amber, clear solution was stirred magnetically at 25°C for 6 h. The reaction mixture was filtered and injected into a RP-HPLC to purify Compounds **4** and **5**. The product fractions of **4** (456 mg, 38% yield) and **5** (384 mg, 32% yield) were dried as white powders.

Syntheses of (+)-*Cis*-Isoalliin (6) and (−)-*Cis*-Isoalliin (7)

Cis-isoalliins (**6** and **7**) were prepared by oxidation of the *cis*-deoxyisoalliin (**4**) with H_2O_2, following the procedure of Yu et al.,[25] with modifications. To a 4-mL aqueous solution of Compound **4** (100 mg, 0.6 mmol) at 0°C, 30% H_2O_2 (60 μL, 0.6 mmol) was added. The mixture was stirred at 20°C for 14 h. The reaction mixture was filtered and injected into a RP-HPLC to purify Compounds **6** and **7**. The product fractions of **6** (47 mg, 43% yield) and **7** (45 mg, 41% yield) were dried as white powders.

Syntheses of (+)-*Trans*-Isoalliin (8) and (−)-*Trans*-Isoalliin (9)

Trans-isoalliin (**8** and **9**) were synthesized using the same procedure as for the synthesis of Compounds **6** and **7**, except that Compound **4** (*cis*-deoxyisoalliin) was replaced



SCHEME 1

Reagents and conditions: (A) NaOH, H_2O, 20°C, 14 h, RP-HPLC; (B) KO*t*-Bu, DMSO, 25°C, 6 h, RP-HPLC; (C) 3% H_2O_2, H_2O, 20°C, 14 h, RP-HPLC.

by Compound **5** (*trans*-deoxyisoalliin). After completion, the reaction mixture was filtered and injected into a RP-HPLC to purify Compounds **8** and **9**. The product fractions of **8** (48 mg, 43% yield) and **9** (43 mg, 39% yield) were dried as white powders.

RESULTS

Scheme 1, shows our pathway of synthesizing (+)-*trans*-isoalliin (**8**). The synthesis starts with the formation of deoxyalliin (**3**) from the commercially available allyl bromide (**1**) and hydrochloride of L-cysteine (**2**).[24,26,27] RP-HPLC is used here to purify Compound **3** directly from the reaction mixture. Compound **3** is eluted at 11.8 min (Fig. 1), and dried as a white powder in 76% yield. The characterization data of Compound **3** are consistent with the literature results[16,26,28–30]: TLC, retention factor (R_f) = 0.68; $[\alpha]^{25}_D$ = −10.4° (c=1, H_2O); ESI-HRMS [MH^+, mass:charge ratio (m/z)], 162.0589 (calculated), 162.0584 (found); RP-HPLC [retention time (t_R)], 11.8 min; ^{1}H NMR (900 MHz, D_2O; Fig. 2), δ = 2.97 [dd, coupling constant (J)=7.6, 14.9 Hz, 1 H, $=CHCH_2SC\underline{H}_2$], 3.07 (dd, J=4.2, 14.9 Hz, 1 H, $=CHCH_2SC\underline{H}_2$), 3.22 (dd, J=7.3, 13.8 Hz, 1 H, $=CHC\underline{H}_2S$), 3.24 (dd, J=7.1, 13.8 Hz, 1 H, $=CHC\underline{H}_2S$), 3.91 (dd, J=4.3, 7.7 Hz, 1 H, $SCH_2C\underline{H}N$), 5.21 (ddd 1 H, J=1.3, 2.2, 9.9 Hz, $C\underline{H}_2=CH$), 5.23 (ddd, 1 H, J=1.3, 2.8, 17.1 Hz, $C\underline{H}_2=CH$), 5.84 (ddq, 1 H, J=9.9, 17.1 , 7.2 Hz, CH2=C$\underline{H}$-S); ^{13}C NMR [distortionless enhancement by polarization transfer with retention of quaternaries (DEPTQ), 225 MHz, D_2O; Fig. 2], δ = 30.05 (1 C, $=\underline{C}HCH_2S$), 33.30 (1 C, $=CH\underline{C}H_2S$), 52.88 (1 C, $CH_2=\underline{C}H$), 117.81 (1 C, $\underline{C}H_2=CH$), 132.91 (1 C, $SCH_2\underline{C}HN$), 172.32 (1 C, $O\underline{C}=O$).

Compounds **4** and **5** are synthesized by base-catalyzed isomerization of Compound **3** with KO*t*-Bu in DMSO at 20°C. The reaction produces 54:46 of Compounds **4** and **5** as expected,[31] which can then be separated by RP-HPLC. Compounds **4** and **5** are eluted at 20.7 and 22.7 min (Fig. 1) and dried as white powders in a total of 70% yield. The characterization data of Compound **4** are consistent with the literature results[15–17]: TLC, R_f= 0.68; $[\alpha]^{25}_D$ = +13.2° (c=1, H_2O); ESI-HRMS (MH^+, m/z), 162.0589 (calculated), 162.0584 (found); RP-HPLC (t_R, min), 20.7; ^{1}H NMR (900 MHz, D_2O; Fig. 3), δ = 1.71 (dd, J=1.5, 6.6 Hz, 3 H, $C\underline{H}_3CH=$), 3.20 (dd, J=7.4, 15.1 Hz, 1 H, $CHSOC\underline{H}_2$), 3.29 (dd, J=4.1, 15.1 Hz, 1 H, $CHSOC\underline{H}_2$), 3.95 (dd, J=4.1, 7.4 Hz, 1 H, $C\underline{H}NH_2$), 5.82 (dq, J=9.2, 6.7 Hz, 1 H, $CH_3C\underline{H}=$), 5.99 (dq, 1 H, J=9.2, 1.5 Hz, $CH=C\underline{H}S$); ^{13}C NMR (DEPTQ, 225 MHz, D_2O; Fig. 3), δ = 13.42 (1 C, $\underline{C}H_3CH=$), 33.26 (1 C, $S\underline{C}H_2CHN$), 52.59 (1 C, $CH=\underline{C}HS$), 122.03 (1 C, $CH_3\underline{C}H=CH$), 127.33 (1 C, $SCH_2\underline{C}HN$), 171.93 (1 C, $O\underline{C}=O$). The characterization data of Compound **5** are consistent with the literature results[20,21]: TLC, R_f = 0.68; $[\alpha]^{25}_D$ = +12.2° (c=1, H_2O); ESI-HRMS (MH^+, m/z), 162.0589 (calculated), 162.0584 (found); RP-HPLC (t_R, min), 22.7; ^{1}H NMR (900 MHz, D_2O; Fig. 4), δ = 1.75 (d, J=5.4 Hz, 3 H, $C\underline{H}_3CH=$), 3.07 (dd, J=8.1, 15.0 Hz, 1 H, $CHSOC\underline{H}_2$), 3.26 (dd, J=3.9, 15.0 Hz, 1 H, $CHSOC\underline{H}_2$), 3.93 (dd, J=3.9, 8.1 Hz, 1 H, $C\underline{H}NH_2$), 5.96 (dq, J=15.0, 5.4 Hz, 1 H, $CH_3C\underline{H}=$), 5.98 (d, 1 H, J=15.0 Hz, $CH=C\underline{H}S$); ^{13}C NMR (DEPTQ, 225 MHz, D_2O; Fig. 4), δ = 17.14 (1 C, $\underline{C}H_3CH=$), 32.93 (1 C, $S\underline{C}H_2CHN$), 53.09 (1 C, $CH=\underline{C}HS$), 119.38 (1 C, $CH_3\underline{C}H=CH$), 131.73 (1 C, $SCH_2\underline{C}HN$), 172.09 (1 C, $O\underline{C}=O$).

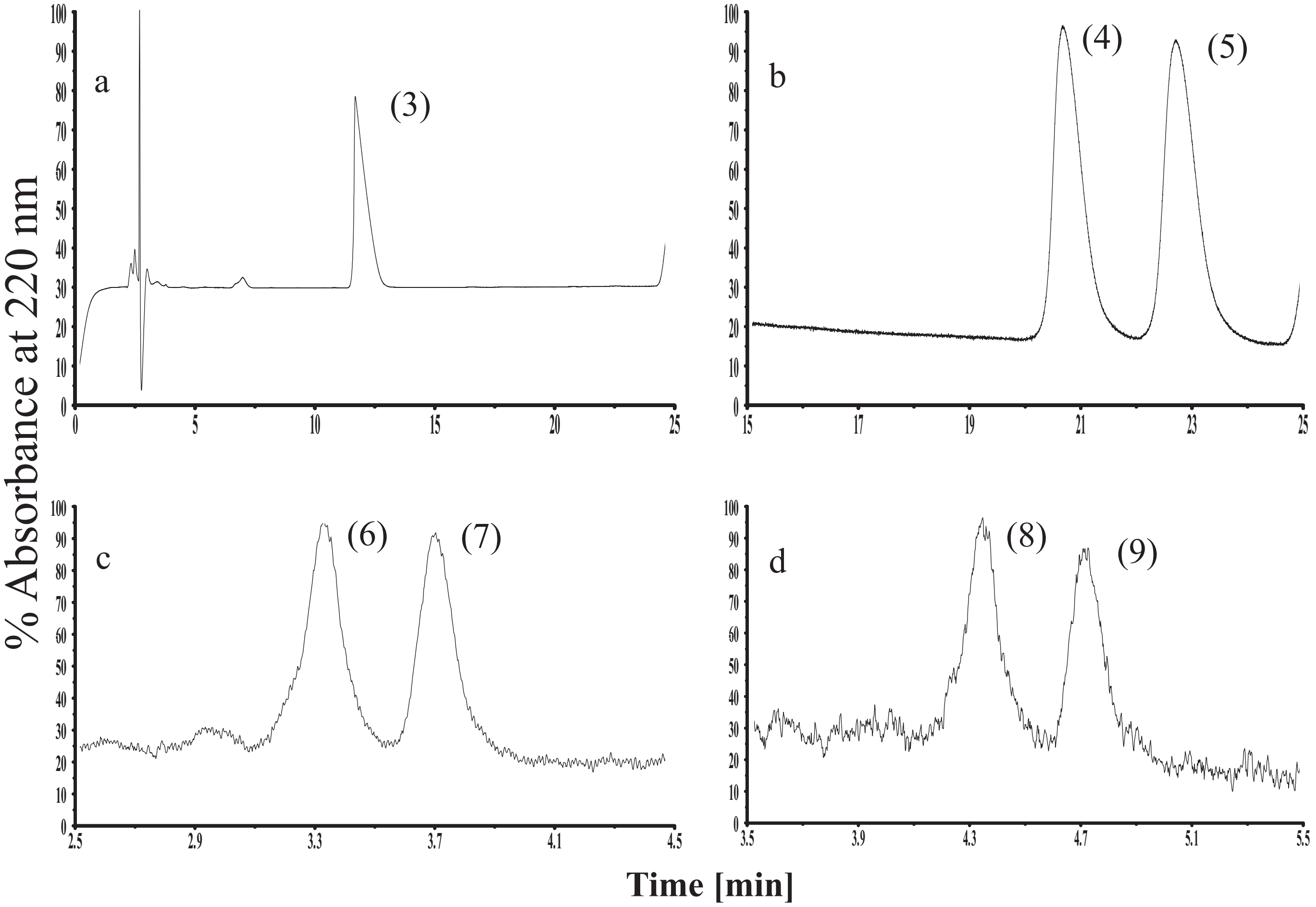


FIGURE 1

RP-HPLC chromatographs of Compounds **3–9**. HPLC was done under the following conditions: instrument, Hewlett Packard 1100 (Agilent Technologies); column, Kinetex RP-C18 (Phenomenex), 5 μm, 250 × 4.6 mm; Solvent A, 0.1% TFA in H_2O; Solvent B, 0.1% TFA in acetonitrile; flow rate, 1 mL/min; elution, a gradient from 100% A for 20 min with linear increase to 100% B for another 5 min; detection wavelength, 220 nm.

Cis-isoalliins (**6** and **7**) and *trans*-isoalliins (**8** and **9**) are prepared by oxidation of the corresponding *cis*-deoxyisoalliin (**4**) and *trans*-deoxyisoalliin (**5**) with H_2O_2 in H_2O, respectively.[20,21,25] The reaction is very efficient in high yield. RP-HPLC is used to separate Compounds **6** from **7** and **8** from **9**. Results show that Compounds **6**, **7**, **8**, and **9** are eluted at 3.3, 3.7, 4.3, and 4.7 min, respectively (Fig. 1). The purified compounds are dried as white powders in a total of 84% yield for Compounds **6** and **7** and a total of 82% yield for Compounds **8** and **9**. The characterization data of Compound **6** agree with the literature results[15–17]: TLC, $R_f = 0.36$; $[\alpha]^{25}_D = +110.4^\circ$ (c=1, H_2O); ESI-HRMS (MH^+, m/z), 178.0537 (calculated), 178.0535 (found); RP-HPLC (t_R, min), 3.3; ^1H NMR (900 MHz, D_2O; Fig. 5), δ = 2.03 (dd, J=1.7, 7.2 Hz, 3 H, $C\underline{H}_3CH=$), 3.38 (dd, J=8.1, 14.3 Hz, 1 H, $CHSOC\underline{H}_2$), 3.54 (dd, J=4.0, 14.3 Hz, 1 H, $CHSOC\underline{H}_2$), 4.26 (dd, J=4.0, 8.1 Hz, 1 H, $C\underline{H}NH_2$), 6.48 (dq, 1 H, J=9.7, 1.9 Hz, $CH=C\underline{H}SO$), 6.67 (dq, J=9.7, 7.2 Hz, 1 H, $CH_3C\underline{H}=$); ^{13}C NMR (DEPTQ, 225 MHz, D_2O; Fig. 5), δ = 14.45 (1 C, $\underline{C}H_3CH=$), 49.19 (1 C, $CH=\underline{C}HSO$), 50.80 (1 C, $OS\underline{C}H_2CHN$), 129.54 (1 C, $CH_3\underline{C}H=CH$), 144.01 (1 C, $SCH_2\underline{C}HN$), 170.18 (1 C, $O\underline{C}=O$). The characterization data of Compound **7** agree with the literature results[15–17]: TLC, $R_f = 0.36$; $[\alpha]^{25}_D = -100.3^\circ$ (c=1, H_2O); ESI-HRMS (MH^+ m/z), 178.0537 (calculated), 178.0535 (found); RP-HPLC (t_R, min), 3.7; ^1H NMR (900 MHz, D_2O; Fig. 6), δ = 2.05 (dd, J=1.8, 7.1 Hz, 3 H, $C\underline{H}_3CH=$), 3.41 (dd, J=7.6, 14.0 Hz, 1 H, $CHSOC\underline{H}_2$), 3.46 (dd, J=5.8, 14.0 Hz, 1 H, $CHSOC\underline{H}_2$), 4.25 (dd, J=5.8, 7.6 Hz, 1 H, $C\underline{H}NH_2$), 6.46 (dq, 1 H, J=9.6, 1.8 Hz, $CH=C\underline{H}SO$), 6.67 (dq, J=9.6, 7.1 Hz, 1 H, $CH_3C\underline{H}=$); ^{13}C NMR (DEPTQ, 225 MHz, D_2O; Fig. 6), δ = 14.39 (1 C, $\underline{C}H_3CH=$), 49.41 (1 C, $CH=\underline{C}HSO$), 51.04 (1 C, $OS\underline{C}H_2CHN$), 130.30 (1 C, $CH_3\underline{C}H=CH$), 143.93 (1 C, $SCH_2\underline{C}HN$), 170.08 (1 C, $O\underline{C}=O$). Compound

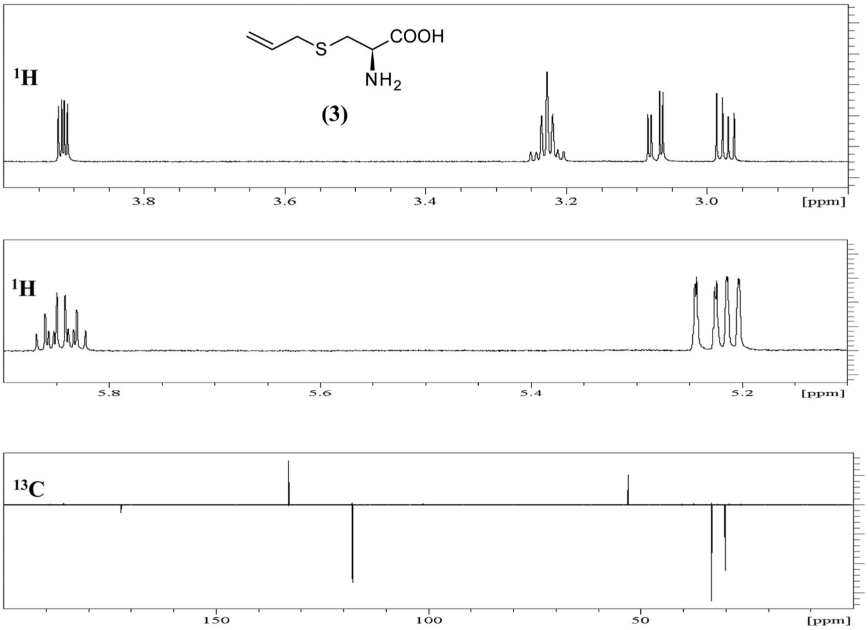


FIGURE 2

^{1}H and ^{13}C NMR spectra of Compound **3**.

8 is confirmed by comparing the characterization data with the literature results[12–14,18,20,21,32,33]: TLC, $R_f = 0.41$; $[\alpha]^{25}{}_D = +74.4°$ (c=1, H_2O); ESI-HRMS (MH^+ m/z), 178.0537 (calculated), 178.0535 (found); RP-HPLC (t_R, min), 4.3; ^{1}H NMR (900 MHz, D_2O; Fig. 7),, $\delta = 1.97$ (dd, J=1.6, 6.9 Hz, 3 H, $C\underline{H}_3CH{=}$), 3.32 (dd, J=7.7, 14.2 Hz, 1 H, $CHSOC\underline{H}_2$), 3.51 (dd, J=5.6, 14.2 Hz, 1 H, $CHSOC\underline{H}_2$), 4.26 (dd, J=5.6, 7.7 Hz, 1 H, $C\underline{H}NH_2$), 6.56 (dq, 1 H, J=15.1, 1.6 Hz, $CH{=}C\underline{H}SO$), 6.74 (dq, J=15.1, 6.9 Hz, 1 H, $CH_3C\underline{H}{=}$); ^{13}C NMR (DEPTQ, 225 MHz, D_2O; Fig. 7), $\delta = 16.95$ (1 C, $\underline{C}H_3CH{=}$), 49.33 (1 C, $CH{=}\underline{C}HSO$), 51.36 (1 C, $OS\underline{C}H_2CHN$), 129.74 (1 C, $CH_3\underline{C}H{=}CH$), 143.27 (1 C, $SCH_2\underline{C}HN$), 170.09 (1 C, $O\underline{C}{=}O$). Compound **9** is confirmed by comparing the characterization data with the literature results[20,21,32]: TLC, $R_f = 0.41$; $[\alpha]^{25}{}_D = -73.2°$ (c=1, H_2O); ESI-HRMS (MH^+ m/z), 178.0537 (calculated), 178.0535 (found); RP-HPLC (t_R, min), 4.7; ^{1}H NMR (900 MHz, D_2O; Fig. 8), $\delta = 1.99$ (dd, J=1.6, 6.9 Hz, 3 H, $C\underline{H}_3CH{=}$), 3.42 (dd, J=3.6, 14.7 Hz, 1 H, $CHSOC\underline{H}_2$), 3.47 (dd, J=8.6, 14.7 Hz, 1 H, $CHSOC\underline{H}_2$), 4.32 (dd, J=3.5, 8.6 Hz, 1 H, $C\underline{H}NH_2$), 6.52 (dq, 1 H, J=15.1, 1.6 Hz, $CH{=}C\underline{H}SO$), 6.71 (dq, J=15.1, 6.9 Hz, 1 H, $CH_3C\underline{H}{=}$); ^{13}C NMR (DEPTQ, 225 MHz, D_2O; Fig. 8), $\delta =$ 16.88 (1 C, $\underline{C}H_3CH{=}$), 48.82 (1 C, $CH{=}\underline{C}HSO$), 50.03 (1 C,

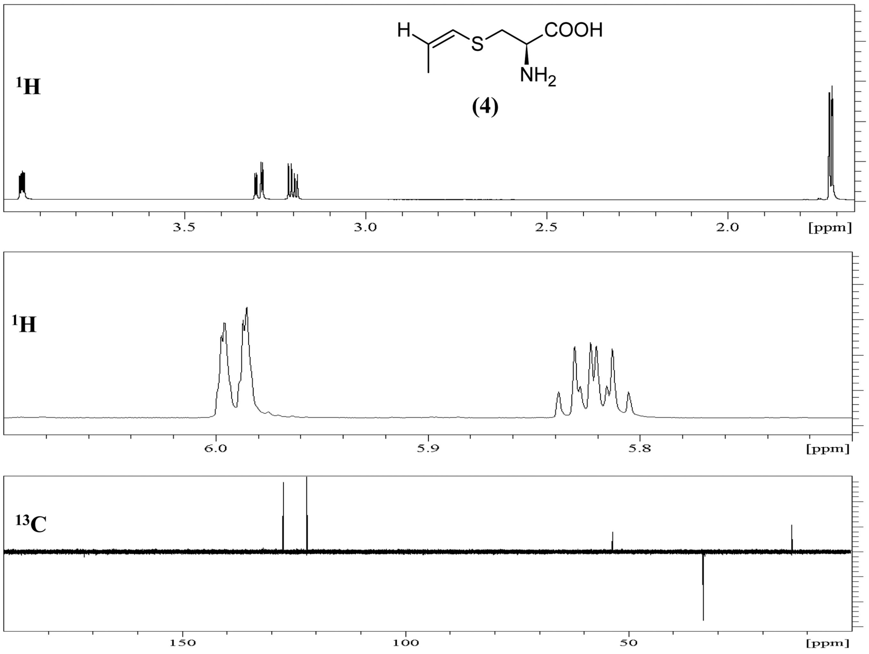


FIGURE 3

^{1}H and ^{13}C NMR spectra of Compound **4**.

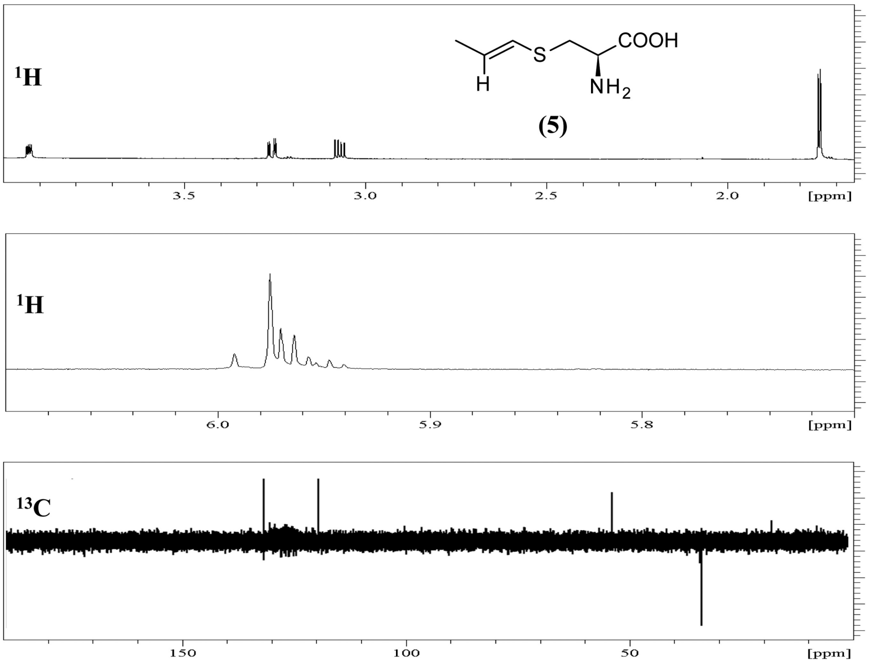


FIGURE 4

^{1}H and ^{13}C NMR spectra of Compound **5**.

$OS\underline{C}H_2CHN$), 128.44 (1 C, $CH_3\underline{C}H{=}CH$), 142.39 (1 C, $SCH_2\underline{C}HN$), 170.28 (1 C, $O\underline{C}{=}O$). It is of interest to note that attempt to produce (+)-*trans*-isoalliin (**8**) by base-catalyzed isomerization of *trans*-S-2-propenyl-L-cysteine sulfoxide (commercially available) failed as a result of the presence of the sulfoxide group.

DISCUSSION

RP-HPLC has been used successfully in purifying organosulfur compounds from *Allium* plants, and is used in this study to separate and purify Compounds **3–9**.[22,34,35] Although allyl chloride is as effective as allyl bromide at a basic condition to synthesize deoxyalliin (**3**), allyl bromide is used in this study. After the reaction is complete, RP-HPLC is used to purify Compound **3** directly from the reaction mixture. This eliminates neutralization with acetic acid, solvent extraction, and crystallization in 95% ethanol steps. Compound **3** is the only compound among Compounds **3–9** that does not show NMR signal for the methyl group as expected. It is interesting that some of the proton-coupling patterns of Compounds **3–9** require nonfirst-order correction, just as in the case of allicin.[34–36] The spin-coupling patterns between δ 2.97 and δ 3.07 and

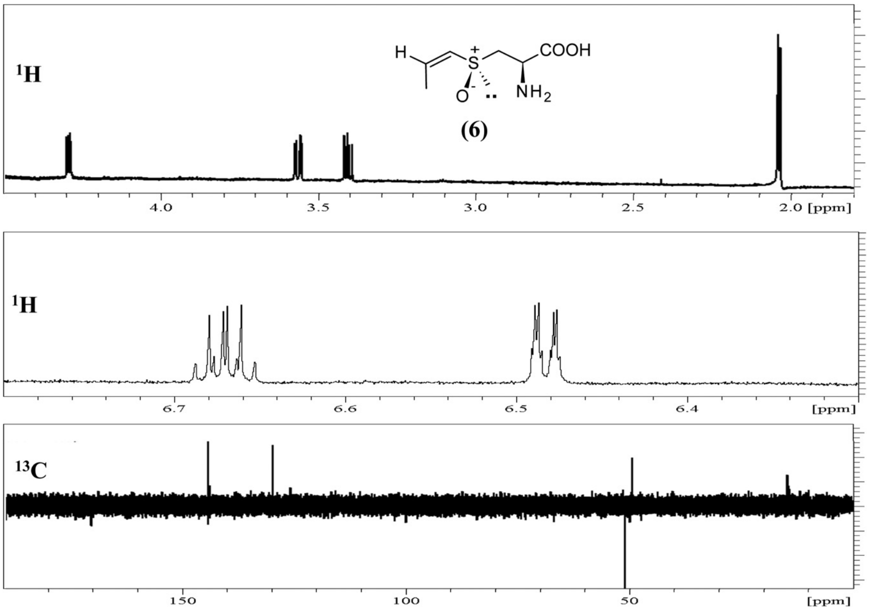


FIGURE 5

^{1}H and ^{13}C NMR spectra of Compound **6**.

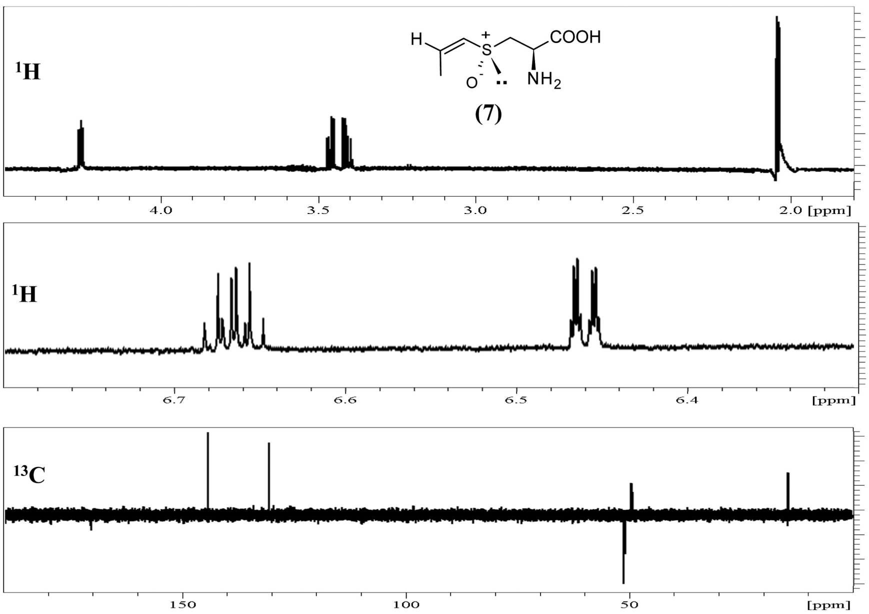


FIGURE 6

^{1}H and ^{13}C NMR spectra of Compound **7**.

between δ 3.22 and δ 3.24 of Compound **3** can be understood by nonfirst-order correction patterns, resulting from the ratios between the chemical shift difference and the spin coupling in Hz of 6.0 ($\Delta\ \nu/J=89.4/14.9$) and 1.5 ($\Delta\ \nu/J=21.0/13.8$), respectively.

Some of the previous studies had reported that base-catalyzed isomerization of deoxyalliin (**3**) with KO*t*-Bu produced *cis*-deoxyisoalliin (**4**) exclusively.[12,15–17] However, several papers in literature reported that this reaction produced a mixture of Compounds **4** and **5** with limited experimental details.[6,22,23] When we duplicated the reaction in our laboratory, 54:46 of Compounds **4** and **5** were produced as expected. The diastereospecific mechanism proposed previously for the high *cis* specificity (>95%) in base-catalyzed isomerization of allyl to propenyl ethers may not be suitable for this reaction.[37] A different mechanism that consists of a carbanion-intermediate state, considerably less stereospecificity as a result of the replacement of oxygen with sulfur, proposed for the base-catalyzed isomerization of allyl to propenyl sulfides (produces 60–65% *cis* isomer), may be more correct.[31,38] A nonfirst-order correction pattern resulting from the ratio between the chemical-

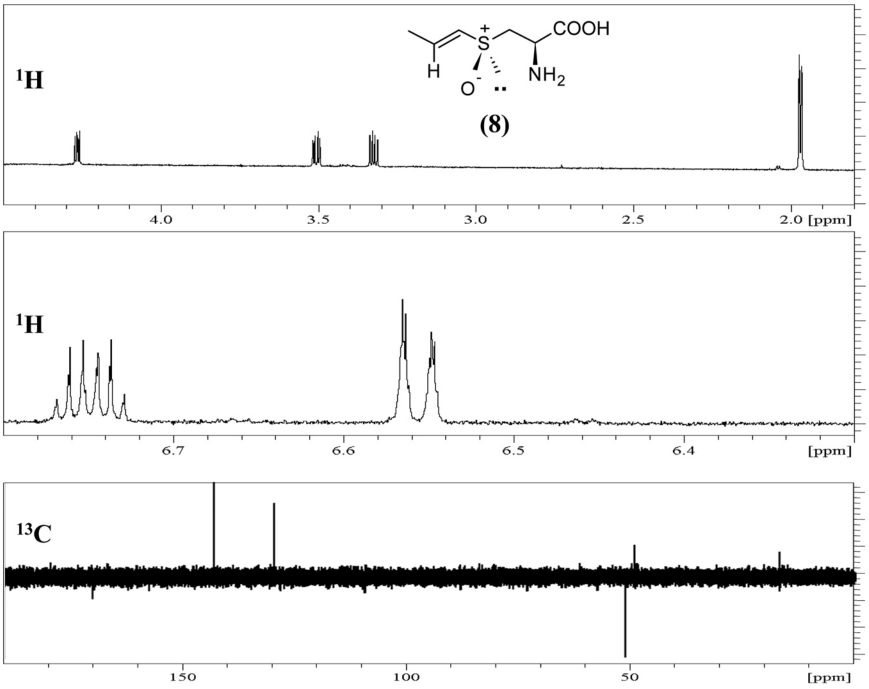


FIGURE 7

^{1}H and ^{13}C NMR spectra of Compound **8**.

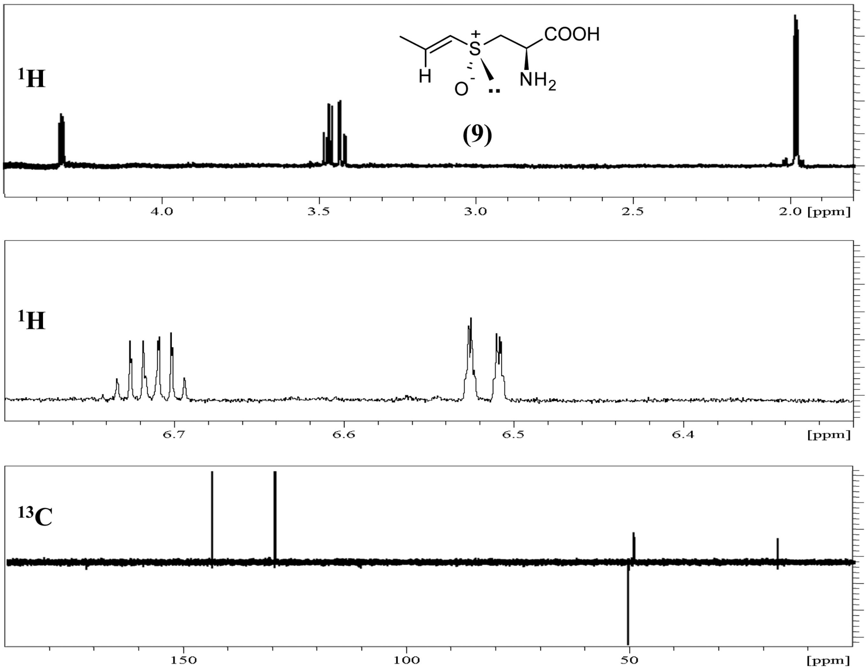


FIGURE 8

^{1}H and ^{13}C NMR spectra of Compound **9**.

shift difference and the spin coupling of 5.6 (Δ ν/J=84.9/15.1) can be used to realize the spin-coupling pattern between δ 3.20 and δ 3.29 of Compound **4**. The spin-coupling patterns between δ 3.07 and δ 3.26 and between δ 5.96 and δ 5.98 of Compound **5** agree with nonfirst-order correction patterns, resulting from the ratios between the chemical shift difference and the spin coupling of 11.2 (Δ ν/J=168.7/15.0) and 1.5 (Δ ν/J=22.4/15.0), respectively. In addition, NMR analysis showed that the two olefinic protons' coupling constants (vicinal coupling) are 9.2 Hz and 15 Hz for Compound **4** and Compound **5,** confirming a *cis* conformation in Compound **4** and a *trans* conformation in Compound **5.** The successful production of Compound **5** facilitates the production of the naturally occurring isoalliin (**8**).

Isoalliins (**6–9**) are produced here by oxidizing deoxyisoalliins (**4** and **5**) with H_2O_2 in H_2O. By careful control of the temperature and concentration, further oxidation to sulfate is avoided. This oxidation reaction converts the divalent sulfur of Compounds **4** and **5** to a trivalent sulfur, which when surrounded by three different groups, becomes a chiral center and produces a mixture of (±) diastereomers, as the oxygen can be introduced from either side of the sulfur. As the chirality of the chiral carbon of the isoalliin is identical but that of the chiral sulfur opposite, Compounds **6** and **7** and Compounds **8** and **9** are (±) diastereomers. As diastereomers are different compounds rather than mirror images, they can be separated by physical and/or chemical methods. Namyslo and Stanitzek[20] and Lee et al.[21] did synthesize (±)-*trans*-isoalliin but failed to produce pure (+)-*trans*-isoalliin. Namyslo and Stanitzek[20] went on to suggest that enantioselective sulfur oxidation, such as modified Sharpless epoxidation or other techniques, can be used to synthesize the naturally occurring (+)-*trans*-isoalliin.[39,40] Instead of naturally occurring (+)-*trans*-isoalliin, Carson and Boggs[15] have reported the separation of the (±) diastereomers of non-natural *cis*-isoalliin by fractional crystallization in 95% ethanol. In our laboratory, instead of fractional crystallization in 95% ethanol, RP-HPLC was used to separate **6** from **7** and **8** from **9**. The (±) diastereomers **6** and **7** show the characteristic smaller NMR vicinal coupling of the *cis*-olefinic protons (J=9.7 Hz), and (±) diastereomers **8** and **9** show the characteristic larger NMR vicinal coupling of the *trans*-olefinic protons (J=15.1 Hz), as expected. In addition, there are differences in NMR chemical shifts between (+) and (−) diastereomers of isoalliin, such as an 18-Hz downfield shift of the terminal methyl protons, a 117-Hz decrease in chemical shift difference between $CHSOC\underline{H}_2$ and $C\underline{H}NH_2$, and a 18-Hz increase in chemical shift difference between $CH_3C\underline{H}=$ and $CH=C\underline{H}S$ for (−) diastereomers (**7** and **9**). The spin coupling patterns between δ 3.38 and δ 3.54 of Compound **6** and between δ 3.41 and δ 3.46 of Compound **7** can be explained by nonfirst-order correction patterns, resulting from the ratios between the chemical-shift difference and the spin coupling of 9.9 (Δ ν/J=142.1/14.3) and 3.3 (Δ ν/J=46.6/14.0), respectively. The spin-coupling patterns between δ 3.32 and δ 3.51 of Compound **8** and between δ 3.42 and δ 3.47 of Compound **9** agree with nonfirst-order correction patterns, resulting from the ratios between the chemical shift difference and the spin coupling of 11.5 (Δ ν/J=163.6/14.2) and 2.8

($\Delta \nu/J=40.7/14.7$), respectively. It is of interest to note that Carson and Boggs[15] reported overlapping TLC spots for (+) and (−) *cis*-isoalliin, with the (−) diastereomer having a slightly smaller R_f value. However, we see the same R_f value for (+) and (−) *cis*-isoalliin, which may be a result of a different developing solvent and TLC plate used in this study.

In summary, we describe here a simple and efficient pathway to synthesize naturally occurring (+)-*trans*-isoalliin, the major cysteine sulfoxide in onions (*Allium cepa*), for the first time in three simple steps. Commercially available L-cysteine and allyl bromide are used as the starting materials in the synthesis. No special thiol-coupling Pd catalyst or Na metal in liquid NH_3 is needed in the reaction scheme. In addition, no fractional crystallization, neutralization, or solvent extraction is needed in this study. This method relies on the high resolving power of RP-HPLC to separate and purify Compounds **3–9**. A study using (+)-*trans*-isoalliin as the key material to shed light on the pigment structures of garlic green/blue or onion pink discoloration is in progress. Further research in replacing L-cysteine with D-cysteine as the starting material[28] and enantioseparation using porous graphitic carbon[41] and/or chiral HPLC columns[42,43] is also in progress. As core facilities are challenged constantly with all kinds of synthesis projects, this work demonstrates that chemical/biochemical techniques are invaluable in fulfilling this demand.

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