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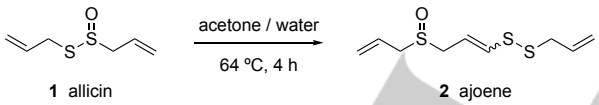
Short Total Synthesis of Ajoene

Filipa Silva,[a] Shaista S. Khokhar,[b] Danielle M. Williams,[b] Robert Saunders,[b] Gareth J. S. Evans,[b] Michael Graz,[b] and Thomas Wirth*[a]

Abstract: We describe a short total synthesis of ajoene, a major biologically active constituent of garlic. The instability of allicin as the only other known alternative starting material has led to the development of a reliable protocol for the synthesis of ajoene from simple building blocks also suitable for upscale operations.

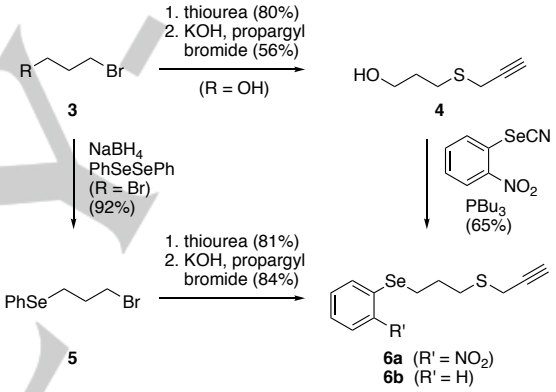
For a long time, garlic extracts and garlic-based products have been used worldwide not only as food ingredients, but also as medicine for the prevention of stroke, coronary thrombosis, and atherosclerosis, as well as in the treatment of infections and vascular disorders.[1] The therapeutic benefits of garlic are manifold and relate to the high concentrations of organosulfur compounds present in this plant. However, the instability of the major component allicin **1** limits the commercial viability of garlic extracts. Among other constituents of garlic, ajoene **2** derived from allicin is biologically active and more stable.[2]

To our knowledge, there is only one reported synthesis of ajoene **2**. Block *et al.* described the biomimetic thermal rearrangement of allicin in aqueous acetone (Scheme 1),[3] recently this synthesis has been extended to produce a trifluorinated analogue.[4] Although the synthesis is a one-pot conversion, it suffers from low yields (34%) due to the formation reactive sulfur-containing intermediates leading also to side products. This reaction does also not allow the synthesis of structurally modified or substituted analogues. More recently, Hunter *et al.* reported a synthetic route to prepare a range of ajoene derivatives, although this route could not be used to synthesize ajoene **2** itself.[5]



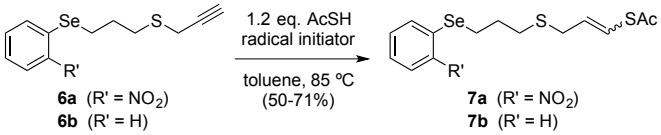
Scheme 1. Block's synthesis of ajoene **2**.

We present here an efficient total synthesis of ajoene **2**. An isothiouronium salt was prepared by reaction of bromide **3** (R = OH) with thiourea, which was then hydrolyzed to the thiol and propargylated to form thioether **4**. The reaction of the hydroxy group in **4** with 2-nitrophenyl selenocyanate and tributylphosphine produced the selenide **6a** (Scheme 2). Alternatively, dibromide **3** (R = Br) can be treated with the phenylselenide anion generated *in situ* from diphenyl diselenide to afford bromide **5**. Compound **5** was then used to synthesize the propargylic thioether **6b** using the same sequence of isothiouronium salt formation, hydrolysis and propargylation. Overall yields for the reaction sequences to **6a** and **6b** are 29% and 63%, respectively. The selenium moiety will serve as the handle to introduce an alkene through a selenoxide elimination.



Scheme 2. Synthesis of aryl propyl selenides **6**.

The next step of the synthesis involved the regioselective addition of thioacetic acid to the terminal alkyne **6**.[6] The reaction was carried out by dissolving alkyne **6** in degassed toluene and heating to 85 °C with a radical initiator added to the solution, followed by the dropwise addition of thioacetic acid over 40 minutes using a syringe pump (Scheme 3). When ACCN [azobis-(cyclohexanecarbonitrile)] was used as radical initiator, compound **7a** was obtained as 2:3 mixture of *E*/*Z* stereoisomers in 50% yield (**7b**: 2:3 *E*/*Z*, 64%). For **7b** the yield was slightly improved to 71% when AIBN [azobis(isobutyronitrile)] was used instead of ACCN. We could show that at this stage the separation of the *E* and *Z* stereoisomers was possible by chromatography. However, the mixture was used in the next reaction, as it is not stereospecific.



Scheme 3. Radical addition of thioacetic acid to form derivatives **7**.

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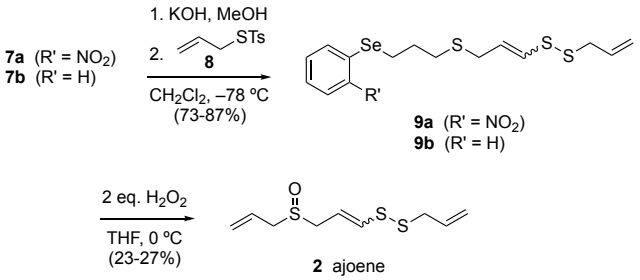
The hydrolysis of **7** to the thioenolate was achieved with potassium hydroxide in methanol and the subsequent sulfenylation with thiosulfonic acid *S*-alkyl ester **8**[7] occurred in good yields to give compound **9**. The reaction was performed at –40 °C in order to avoid side reactions of the highly reactive thioenolate. The reaction with compound **7a** (2:3 mixture of *E*/*Z* stereoisomers) afforded **9a** in 73% yield with the same *E*/*Z* ratio. When the reaction was performed with compound **7b** (1:1 mixture of *E*/*Z* stereoisomers), compound **9b** was obtained in 87% yield and the ratio of *E*/*Z* stereoisomers changed to 2:3. Since the stereoisomers could be separated by chromatography, a reaction with *Z*-**7b** was performed in order to verify if isomerization to the *E*-isomer occurs even at very low temperatures. Indeed, the reaction afforded **9b** as a 3:2 mixture of *E*/*Z* stereoisomers in 85% yield.

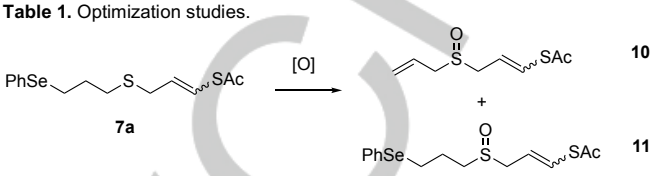


Scheme 4. Synthesis of ajoene **2**.

In the final step of the synthesis, compound **9** is treated with two equivalents of 30% w/w hydrogen peroxide solution to form ajoene **2** in a 2:3 mixture of *E*/*Z* stereoisomers in 27% (**9a**) and 23% (**9b**) yield. The selenide as well as the sulfide functionality is oxidized and while the selenoxide is undergoing a direct selenoxide elimination to form a double bond, the sulfoxide is retained in the product molecule. The *syn*-elimination of alkyl aryl selenoxides is an efficient synthetic procedure to form alkenes. It is known that electron-withdrawing substituents on the aromatic ring increase both the rate of elimination and the yield of the alkene.[8] However, the use of the selenium derivative with an electron-withdrawing substituent **9a** did not show any advantage when compared with compound **9b** as the yields are almost identical. A perselenenic acid byproduct might be able to catalyze the oxidation to the sulfoxide.[9] The yields for the conversion from **9** to ajoene **2** were rather low at a 0.3 mmol scale.

Further optimization studies of the selenoxide elimination and concomitant sulfur oxidation were carried out. For this study, compound **7a** was used as the model substrate to find suitable reaction conditions. Product **10** can also be used as ajoene precursor. Different oxidation conditions were investigated and the results are presented in Table 1. The reaction of compound **7a** with 2 equivalents of H_2O_2 (50% w/w) afforded products **10** (23%) and **11** (19%) (Table 1, entry 1). Increasing the amount of oxidant to 3 or 4 equivalents did not improve the yield of compound **10** and compound **11** was still isolated (Table 1, entries 2 and 3). The complex urea • hydrogen peroxide (UHP) was also used as an alternative to aqueous hydrogen peroxide solution. The reaction of compound **7a** with 2 equivalents of UHP afforded beside compound **10** in 33%, compound **11** in 17% (Table 1, entry 4). Interestingly, when the reaction of compound **7a** was carried out in the presence of $NaIO_4$, compound **11** was isolated as a major compound in 50% yield (Table 1, entry 5). *meta*-Chloroperbenzoic acid (*m*CPBA) was also investigated as a suitable oxidant and found to react giving comparable yields (Table 1, entry 6).

Table 1. Optimization studies.



Entry	Oxidation conditions	Yield [%]		
		7a	**10**	**11**
1	2 equiv. H_2O_2 (50% w/w), THF 0 ºC (1 h) – rt (2 h)	20	23	19
2	3 equiv. H_2O_2 (50% w/w), THF 0 ºC (1 h) – rt (2 h)	21	20	25
3	4 equiv. H_2O_2 (50% w/w), THF 0 ºC (1 h) – rt (2 h)	-	12	9
4	2 equiv. UHP, CH_2Cl_2 0 °C (1 h) – rt (2 h)	6	35	6
5	2 equiv. $NaIO_4$, CH_3OH/H_2O 0 °C (2 h), then rt (6 h)	-	9	50
6	2 equiv. *m*-CPBA, $CHCl_3$ 0 ºC (1 h) – rt (2 h)	-	37	16
7	2 equiv. H_2O_2 (50% w/w), CH_2Cl_2, 1.5 equiv. DIPA, 0 ºC (1 h) – rt (2 h)	20	27	-
8	2 equiv. *m*CPBA, CH_2Cl_2, 2 equiv. DIPA 0 ºC (1 h) – rt (2 h)	-	46	-
9	2 equiv. *m*CPBA, CH_2Cl_2, 2 equiv. DIPA 0 ºC (1 h) – rt (24 h)	-	44	-

Independently of the reaction conditions, compounds **10** and **11** were the two major products formed and isolated. However, smaller amounts of other non-identified side products were also detected. Areneselenenic acid generated during the selenoxide elimination is in equilibrium with its disproportionation products (diaryl diselenide and areneseleninic acid). Under neutral or acidic conditions they can react with alkenes leading to the formation of side products. Side reactions can be suppressed by addition of alkyl amines. When 1.5 equivalents of diisopropylamine (DIPA) was added to the reaction with H_2O_2, the formation of compound **11** was suppressed, but compound **10** was only isolated in 27% yield. Adding 2 equivalents of DIPA to the reaction with *m*CPBA not only stopped the formation of compound **11**, but also improved the yield of product **10** to 46% (Table 1, entry 8). The same reaction conditions with a longer reaction time did not affect the yield of compound **10** (Table 1, entry 9).

The complete synthesis of ajoene **2** was scaled up with slightly different results being obtained.[10] The synthesis of **5** proceeded with 58% yield on a 4 mol scale, while the subsequent thiol formation and propargylation led to **6b** in 87% (2.9 mol). Radical addition of thioacetic acid proceeded similarly well compared to

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the small-scale synthesis (**7b**: 75%, 1.4 mol) as did the thioacetate cleavage and thioallylation to **9b** (74%, 1.1 mol). The final oxidation to ajoene **2** had superior yields (65%) as compared to the small scale synthesis and 169 g (0.72 mol) of ajoene **2** was isolated in ~90% purity as determined by HPLC and NMR analysis.

Much of the research interest in ajoene **2** resides in its biological activity. It has been shown to have efficacy in a number of biological studies that include antithrombotic and antifungal activities.[11] In order to further evaluate **2**, its activity in a biological assay was also examined. Ajoene's ability to act as a quorum sensing inhibitor (QSI) was selected, as this is one of its more recent remarkable biological properties. Quorum sensing (QS) is a mechanism of cell-cell communication in bacteria facilitated by the secretion and detection of signalling molecules such as *N*-acyl homoserine lactones in Gram-negative bacteria.[12] QS allows bacteria to synchronise specific gene expression which has an impact on their pathogenicity and is thought to have a significant role in the formation of biofilms. Recent studies have shown that ajoene **2** is an effective QS inhibitor against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and could be utilised for the treatment of chronic biofilm infections by exploiting the QS system.[13] In this study, we employed a reporter strain (PaO1-lasB-gfp)[13a] whereby QS gene expression was monitored over time in response to ajoene treatment.

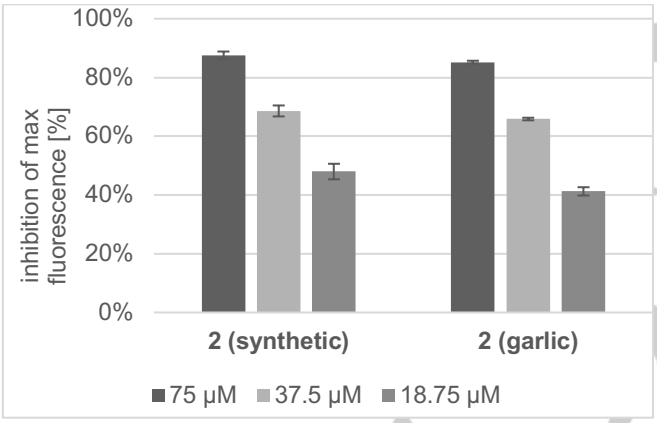

Figure 1. Inhibition of a PaO1 *lasB-gfp* reporter strain, where inhibition of fluorescence is directly related to QS-controlled expression,

Two ajoene products were examined; **2 (synthetic)** as synthesized above and ajoene **2 (garlic)** extracted from garlic using the thermal rearrangement conditions.[3] the results are expressed as a mixture of *E* and *Z* ajoene. Both ajoene samples are effective QSIs as shown by their inhibition of the fluorescence values in Figure 1, where a reduction in fluorescence is directly related to the down regulation of the QS gene *lasB*.

The samples show a very similar pattern of concentration-dependent inhibition. This is reiterated in the IC_{50} calculations where ajoene **2 (garlic)** extracted from garlic had an IC_{50} value of 27.7 µM and synthetic ajoene **2 (synthetic)** had an IC_{50} value of 28.5 µM. The IC_{50} values are comparable between the different origins of ajoene **2**.

In conclusion, we describe an efficient total synthesis of ajoene from easily available starting materials. The simultaneous introduction of the allyl moiety and the sulfoxide in the final step allows a straightforward generation of the target molecule. Up-scaling of the synthetic sequence was possible leading for the first time to the synthesis of synthetic ajoene in larger amounts. Synthetic ajoene and ajoene derived from garlic have been investigated regarding their efficiency as quorum sensing inhibitors.

Experimental Section

The vinyl disulfide **9a** (0.140 g, 0.33 mmol) is dissolved in THF (3 mL) and cooled to 0 °C under N_2, and H_2O_2 (30% w/w in H_2O, 0.075 mL, 0.66 mmol) added dropwise. The mixture was allowed to stir for 1 h at 0 °C and then warmed to rt (2 h). Sat. aq. $NaHCO_3$ (5 mL) was added and the residue was extracted with EtOAc (2 × 10 mL). The combined organic fractions were washed with brine (2 × 10 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the resulting residue purified by column chromatography to afford ajoene **2** (21 mg, 27%, *E/Z* = 1:1.8) as a pale-yellow oil.

Acknowledgements

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Keywords: ajoene • allicin • garlic • organosulfur compounds • selenoxide elimination

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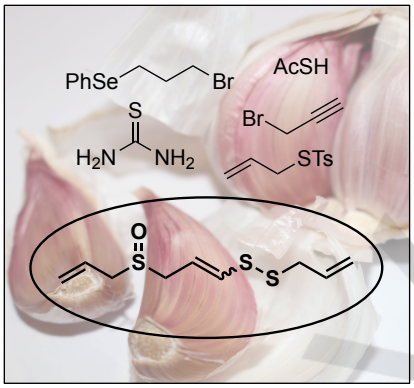
Making garlic: An efficient total synthesis of ajoene from easily available starting materials is reported.



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