# Efficient synthesis of brussalexin A, a remarkable phytoalexin from Brussels sprouts†

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**The synthesis of brussalexin A, the first phytoalexin containing an allyl thiolcarbamate group, and its selective inhibitory activity against fungal plant pathogens is described.**

Plants under stress biosynthesise *de novo* antimicrobial secondary metabolites which are known in general as phytoalexins.[1] Due to a pivotal importance in plant fitness, phytoalexins and their interaction with economically important pathogens are the subject of many ongoing studies.[2] Phytoalexins containing dithiocarbamate or thiolcarbamate functionalities such as brassinin (**1**), brassitin (**2**), 1-methoxybrassinin (**3**), 1-methoxybrassitin (**4**), and 4-methoxybrassinin (**5**) are produced by many species of crucifer plants.[3,4] Coincidentally but worthy of note, dithiocarbamate- and thiolcarbamate-containing compounds have been used for decades in a broad range of pesticides. For example, Mancozeb is a broad spectrum fungicide,[5] Thiobencarb[6] is a widely used herbicide, and Cartap[7] is an insecticide known to block the nicotinic acetylcholine receptor/ion channel complex of insects (Fig. 1).

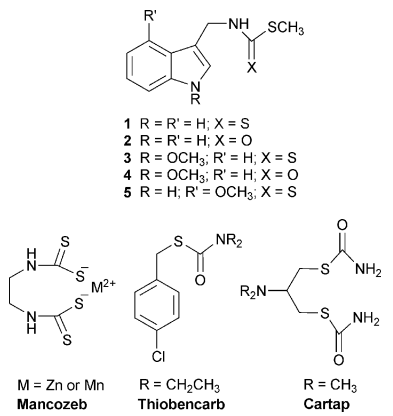
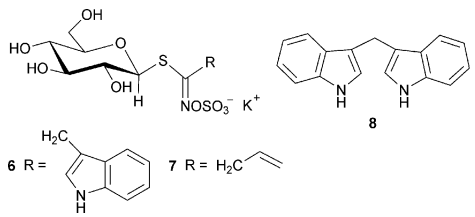



**Fig. 1** Selected phytoalexins (**1–5**) from crucifers and pesticides.

Crucifers are widely studied because of their considerable economical importance as oilseed crops [canola (*Brassica napus* and *B. rapa*)], vegetables [cabbage (*B. oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*) and turnip (*B. rapa* ssp. *rapa*)], and condiments [brown mustard (*B. juncea*)]. Furthermore, convincing evidence of the protective effect of crucifer vegetables against cancer[8] and other physiological problems continue to drive investigations on the production/degradation and role of their secondary metabolites, namely glucosinolates and isothiocyanates.[9]

Brussels sprouts (*B. oleracea* var. *gemmifera*), a vegetable consumed worldwide, are known to produce a great variety of aromatic and aliphatic glucosinolates, including glucobrassicin (**6**) and sinigrin (**7**).[10] In addition, 3,3′-diindolylmethane (**8**) is a naturally occurring product derived from glucobrassicin (**6**).[11] Nonetheless, the phytoalexins of Brussels sprouts have not been reported to date.




In this study, Brussels sprouts under stress were shown to produce a very intriguing thiolcarbamate that we named brussalexin A (**9**). Brussalexin A is the first naturally occurring thiolcarbamate in which the sulfur atom is attached to the 3-methylindolyl moiety. Here we report the first isolation, synthesis, and antifungal activity of this phytoalexin.

Brussels sprouts were irradiated under UV light (elicited) and incubated as described in the ESI†. After 72 hours, tissues were ground and extracted with ethyl acetate. HPLC chromatograms of extracts of elicited sprouts indicated the presence of several peaks not present in control tissues (non-elicited). Several of these peaks corresponded to known phytoalexins; however, one of the peaks ($t_R$ = 17.1 min) found no match in our libraries (DAD and MS).[12] To obtain amounts of this unidentified compound sufficient for structural identification, larger amounts of sprouts were elicited, incubated, ground and extracted. The organic extract was separated by multiple chromatography to yield the unidentified compound (*ca.* 2 mg from 3.9 kg of fresh tissue). The HR-EI-MS‡ of this unknown compound indicated the molecular formula $C_{13}H_{14}N_2OS$; the NMR spectroscopic data§ indicated a monosubstituted indole with a methylene at C-3, an exchangeable proton attributable to N-1 ($C_9H_8N$), and an allyl substituent coupled to an exchangeable proton ($C_3H_5$). The remaining atoms (HCNOS) required to fulfil the molecular formula were used to connect the methylene and allyl groups. Since the allyl group showed coupling to an exchangeable proton, the only possible structural arrangement was deduced to be that shown by structure **9**. This structure was confirmed by synthesis, as described below.

The synthesis of thiolcarbamates has been achieved using a variety of methods. Palladium-catalysed reactions of disulfides with amines and carbon monoxide,[13] reaction of amines with chlorothioformates[14] or with phosgene[15] and thiols are among

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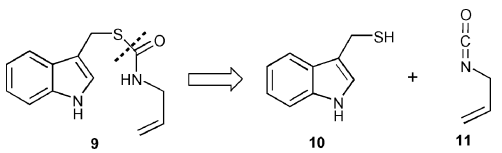
† Electronic supplementary information (ESI) available: Experimental details, synthesis and spectroscopic data of all new compounds (**9** and **15–19**). See DOI: 10.1039/b702156a

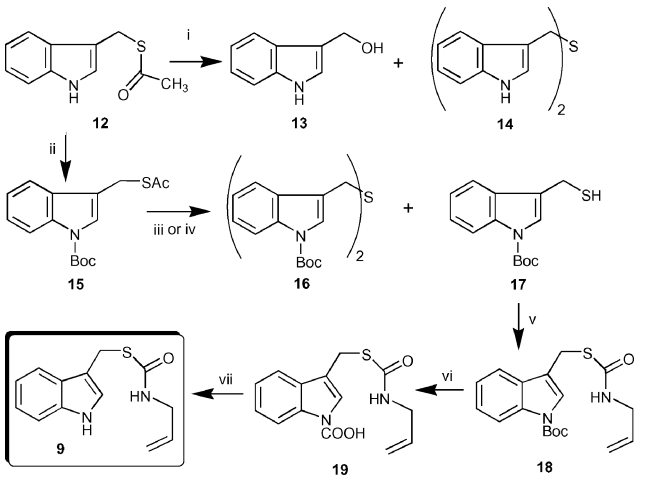
the most frequently used preparative methods. Likely due to the significance of the biological activities of thiolcarbamates, the patented literature has described several efficient preparations as well.[16] In spite of these wide range of methodologies, preparation of a thiolcarbamate by coupling of a thiol with an isocyanate has been used only in a few instances. Nonetheless, this method appeared a reasonable strategy, since the required allyl isocyanate (**11**) was commercially available and indolyl-3-methanethiol (**10**) (Scheme 1) could be obtained by hydrolysis of indolyl-3-methyl thioacetate (**12**).[17]




**Scheme 1** Retrosynthetic analysis of brussalexin A (**9**).

In our hands, however, hydrolysis of indolyl-3-methylthioacetate (**12**) under a variety of conditions yielded diindolyl-3,3′-methylsulfide (**14**) as a major product, along with indolyl-3-carbinol (**13**) (Scheme 2). The key intermediate indolyl-3-methanethiol (**10**) could be obtained only as the *N*-Boc-protected thiol (**17**), in excellent yield (89%) from hydrolysis of **15** with KOH/THF under argon atmosphere and at room temperature. When KOH/THF hydrolysis was carried out at higher temperatures (>50 °C), formation of only bis(1-Boc-indolyl-3-methyl)monosulfide (**16**) was observed (Scheme 2). 1-Boc-indolyl-3-methanethiol (**17**) was stable enough to be characterised spectroscopically, as reported in the ESI†. Next, 1-Boc-indolyl-3-methanethiol (**17**) was coupled with allyl isocyanate (**11**), in KOH/THF at 70 °C, to yield **18** quantitatively. Finally, deprotection of **18** in neat TFA at room temperature yielded **19**, which after solvent removal and heating of the residue at 50 °C for 2 h, afforded compound **9** quantitatively (Scheme 2). In conclusion, compound **9** was efficiently synthesised from indolyl-3-methylthioacetate (**12**), requiring only one chromatographic separation in the last step, in 73% overall yield. Compound **9** was identical in all respects to the natural metabolite isolated from UV-elicited Brussels sprouts.

With compound **9** available in sufficient amounts, its effect on the important fungal crucifer pathogens *Leptosphaeria maculans* (BJ 125, isolate virulent on canola; Laird 2, isolate virulent on mustard), *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Alternaria brassicicola* was determined. Mycelial growth inhibition




**Scheme 2** Synthesis of brussalexin A (**9**). *Reagents and conditions*: i, KOH, THF, argon, rt; ii, (*t*-Boc)$_2$O, 4-DMAP, THF, 60 min, 99% yield; iii, KOH, THF, argon, rt, 4 h, 88% yield; iv, KOH, THF, argon, 70 °C, 4 h, 44% yield; v, allyl isocyanate (**11**), KOH, 70 °C, 20 min, 88% yield; vi, TFA, rt; vii, 50 °C, 2 h, 91%.

bioassays, as described in the ESI†, established that compound **9** displayed a higher inhibitory effect against *S. sclerotiorum*, causing 89% inhibition at $5.0 \times 10^{-4}$ M, than against the other three species (Table 1). Brussels sprouts are susceptible to *L. maculans*, *R. solani* and *A. brassicicola*, and appear to be more tolerant to *S. sclerotiorum*; however, since this is a commercial variety, the degree of disease susceptibility to each species is not known.

Compound **9** is a plant metabolite with antimicrobial activity that is biosynthesised in response to abiotic stress (UV), and is not detectable in non-stressed plants. Consequently, this metabolite is a new phytoalexin, for which we propose the name brussalexin A¶ (**9**).

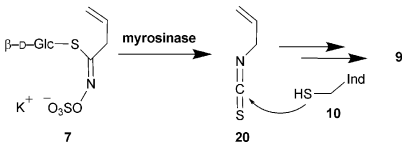
The structure of brussalexin A (**9**) is rather puzzling, as its biosynthetic origin does not appear to fit in the currently known biosynthetic pathway of most crucifer phytoalexins.[3] Production of allyl isothiocyanate (**20**) in crucifers results from hydrolysis of sinigrin (**7**) by a plant enzyme(s) generally known as myrosinase(s) (thioglucoside glucohydrolases, EC 3.2.1.147, Scheme 3),[18] which appears to be the major glucosinolate of Brussels sprouts.[10] Thus it is proposed that brussalexin A (**9**) could result biosynthetically from addition of thiol **10** (likely generated *in situ* due to its high reactivity) to isothiocyanate **20**, followed by desulfurisation.

**Table 1** Inhibitory activity of brussalexin A (**9**) against commercially important plant pathogens: *Leptosphaeria maculans*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Alternaria brassicicola*

| Fungal species | Growth inhibition[a] (%) | | |
|---|---|---|---|
| | At $5 \times 10^{-5}$ M | At $2.5 \times 10^{-4}$ M | At $5 \times 10^{-4}$ M |
| *A. brassicicola*[b] | 30 ± 11 | 47 ± 6 | 56 ± 6 |
| *L. maculans*[b] isolate BJ-125 | 10 ± 1 | 28 ± 7 | 47 ± 5 |
| *L. maculans*[b] isolate Laird-2 | 10 ± 1 | 21 ± 1 | 32 ± 3 |
| *R. solani*[c] | 22 ± 3 | 45 ± 4 | 54 ± 2 |
| *S. sclerotiorum*[d] | 65 ± 2 | 82 ± 2 | 89 ± 3 |

[a] The percentage inhibition was calculated using the following formula: Percentage inhibition = 100 − [(growth on treated/growth in control) × 100]. The results are the mean of at least three independent experiments. [b] Results after 5 days of incubation. [c] Results after 3 days of incubation. [d] Results after 1 day of incubation.




**Scheme 3** Proposed biosynthetic precursors **10** and **20** of brussalexin A (**9**).

Brussels sprouts are cultivated in many regions of the world, and thus are expected to show great variation in the degree of susceptibility to different fungal diseases. Therefore, to determine the significance of brussalexin A (**9**) in defence reactions against pathogenic fungi, it would be necessary to analyse its production in various horticultural regions. Notwithstanding this lack of information, the reactions of the various pathogens to brussalexin A (**9**) are expected to provide further insights into its role in plant defence mechanisms.[2] Finally, considering the uniqueness of the allyl thiolcarbamate group of **9**, it would be of interest to investigate its potential anticarcinogenic and antioxidative properties.

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## Notes and references

‡ HR-EI-MS: calc. for $C_{13}H_{14}N_2OS$ ($M^+$) $m/z$ 246.0826, found 246.0824; EI-MS ($m/z$, %): 246 (9), 130 (100).
§ $^1$H-NMR (500.1 MHz, $CD_3CN$): $\delta$ 9.17 (br s, 1H), 7.62 (d, $J = 8$ Hz, 1H), 7.42 (d, $J = 8$ Hz, 1H), 7.23 (d, $J = 2$ Hz, 1H), 7.18 (dd, $J = 8$ Hz, 1H), 7.09 (dd, $J = 8$ Hz, 1H), 6.48 (br s, 1H), 5.87 (m, 1H), 5.17 (dd, $J =$ 17, 1.5 Hz, 1H), 5.11 (dd, $J = 1.5$, 11 Hz, 1H), 4.36 (br s, 2H), 3.86 (br s, 2H). $^1$H-NMR (500.1 MHz, $CDCl_3$): $\delta$ 8.05 (br s, 1H), 7.69 (d, $J = 8$ Hz, 1H), 7.38 (d, $J = 8$ Hz, 1H), 7.23 (br s, 1H), 7.24 (dd, $J = 8$, 8 Hz, 1H), 7.18 (dd, $J = 8$, 8 Hz, 1H), 5.85 (m, 1H), 5.36 (br s, 1H), 5.23 (d, $J = 17$ Hz, 1H), 5.17 (d, $J = 10$ Hz, 1H), 4.44 (br s, 2H), 3.96 (br s, 2H). $^{13}$C-NMR ($CDCl_3$, 125.8 MHz): $\delta$ 167.5 (s), 136.3 (s), 133.7 (d), 126.6 (s), 123.4 (d), 122.4 (d), 119.8 (d), 119.0 (d), 116.9 (t), 112.3 (s), 111.3 (d), 43.7 (t), 25.5 (t).
¶ The name brussalexin A is proposed because additional elicited compounds appear to be produced in Brussels sprouts, although their structures remain to be determined.

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