JOC Article

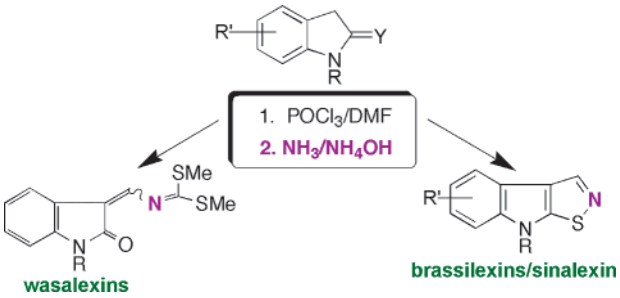
Concise Syntheses of the Cruciferous Phytoalexins Brassilexin, Sinalexin, Wasalexins, and Analogues: Expanding the Scope of the Vilsmeier Formylation

M. Soledade C. Pedras* and Mukund Jha

Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon SK S7N 5C9, Canada

soledade.pedras@usask.ca

Received November 12, 2004

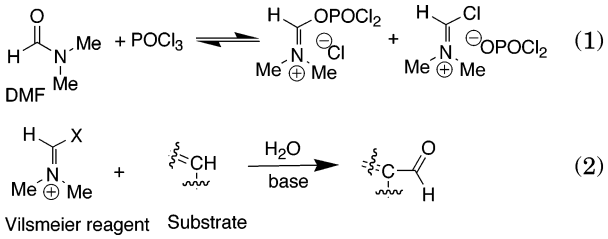


Efficient syntheses of the phytoalexins brassilexin, sinalexin, and analogues are demonstrated through the application of the Vilsmeier formylation to indoline-2-thiones followed by a new aqueous ammonia workup procedure. Similarly, a very concise two-pot synthesis of the phytoalexins wasalexins using sequential formylation−amination of indolin-2-ones is described. Remarkably, this novel aqueous ammonia workup allows the sequential one-pot formylation−amination, expanding substantially the scope of the Vilsmeier formylation of both indoline-2-thiones and indolin-2-ones. The examination of the formylation−amination reaction and optimization of conditions, as well as the syntheses and antifungal activities of several brassilexin analogues, are reported.

Introduction

Since the first publication appeared in 1925, the Vilsmeier−Haack reaction has been applied to an immense variety of substrates, from substituted benzenes to complex heterocycles.[1] The Vilsmeier reagent, an iminium salt with weak electrophilic character, results from the reaction between an acid chloride (e.g., $POCl_3$, $SOCl_2$, $(COCl)_2$, $COCl_2$) and an amide, usually DMF (Scheme 1, eq 1). Further reaction of the Vilsmeier reagent with a reactive aromatic[2] substrate followed by basic workup affords, in general, acylation products via electrophilic aromatic substitution (Scheme 1, eq 2).[1] If DMF is used in the reaction, the product of the Vilsmeier reaction is an aldehyde, thus the Vilsmeier reaction is often called Vilsmeier formylation.

SCHEME 1. Vilsmeier−Haack Reaction[1]



Among the annulated furans, thiophenes and pyrroles, indoles display the highest reactivity. In general, the regiochemistry of the Vilsmeier reaction of indoles (e.g., **1**) is quite predictable, occurring at the 3-positon, unless this position is substituted, as for example in skatole (**2**) (Scheme 2, eqs 3 and 4).[1] Furthermore, substrates such as indolin-2-ones (**6**−**8**) with lower reactivity than indole (**1**) have been formylated using the Vilsmeier reagent to afford the corresponding 2-chloro-3-formyl indoles (**11**) (Scheme 2, eq 5).[1] Until recently, indoline-2-thiones (**9**)

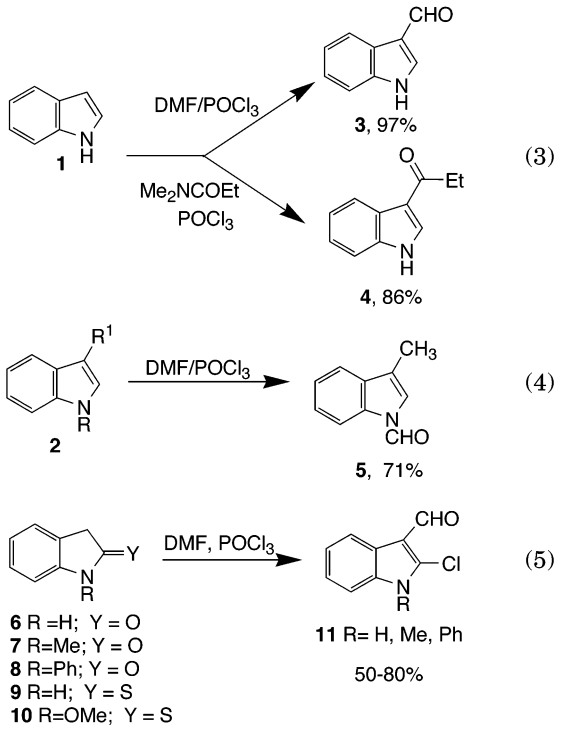
* Address correspondence to this author. Phone: (306)966-4772. Fax: (306)966-4730.

(1) (a) Jones, G.; Stanforth, S. P. In *Organic Reactions*; Paquette L. A., Ed.; Wiley: New York, 1997; Vol. 49, pp 1−330. (b) Downie, I. M.; Earle, M. J.; Heaney, H.; Shuhaibar, K. F. *Tetrahedron* **1993**, *49*, 4015.

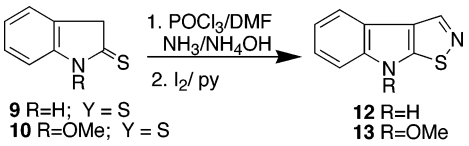
(2) The Vilsmeier formylation has also been applied to alkenes, acetals, and ketals, see: Smith, M. B.; March, J. *Advanced Organic Chemistry*, 5th ed.; J. Wiley & Sons: New York, 2001; p 785 and references therein.

10.1021/jo0479866 CCC: $30.25
Published on Web 02/03/2005

SCHEME 2. Vilsmeier Formylation of Indoles (Eqs 3 and 4) and Indolin-2-ones (Eq 5)[1]

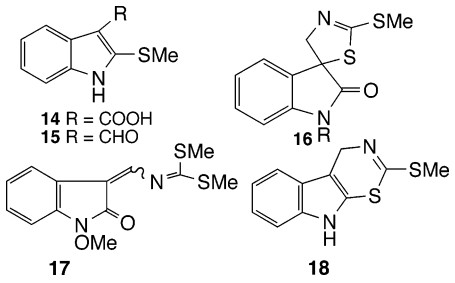


SCHEME 3. Application of the Vilsmeier Formylation to the Synthesis of Brassilexin (12) and Sinalexin (13)



had not been formylated under Vilsmeier conditions,[3] but 2-thioethers such as 2-thiomethylindole have been formylated in good yield.[4] Despite the lack of precedent, the most efficient synthesis of sinalexin (**13**) used the Vilsmeier formylation of 1-methoxyindoline-2-thione (**10**) followed by a new aqueous ammonia workup procedure (Scheme 3). Similarly, although several syntheses of brassilexin (**12**) have been published to date,[5] the most efficient preparation was also accomplished under Vilsmeier conditions (Scheme 3).[3]

Our interest in the formylation of indolin-2-ones (**6**, **7**)[6] and indoline-2-thiones (**9**, **10**)[7] derives from the potential application of these substrates to the synthesis of tryptophan-derived alkaloids, in particular phytoalexins such as brassilexin (**12**),[8] sinalexin (**13**), brassicanate A (**14**),[9] brassicanal A (**15**), spirobrassinins (**16**), wasalexins (**17**), and cyclobrassinin (**18**).[5] Phytoalexins are secondary metabolites biosynthesized de novo by plants in response to diverse forms of stress, including pathogen attack.[10] Brassilexin (**12**) and sinalexin (**13**) are among the most potent phytoalexins produced by economically important cruciferous plants. Sinalexin (**13**) appears to be involved in defense mechanisms of white mustard (*Sinapis alba*),[11] whereas brassilexin (**12**) and wasalexins (**17**) have similar roles in brown mustard (*Brassica juncea*)[12] and wasabi (*Wasabia japonica*),[13] respectively. To investigate phytoalexin metabolic pathways in plants and their fungal pathogens, isotopically labeled phytoalexins are required, hence it is crucial to design concise and efficient syntheses.



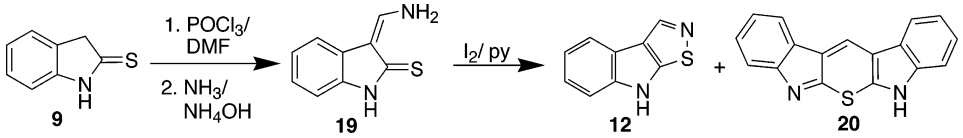
Toward this end, we investigated conditions for the Vilsmeier formylation of indoline-2-thiones (**9**, **23a**−**j**) and indolin-2-ones (**6**−**7b**). Remarkably, the Vilsmeier formylation of these substrates followed by a new aqueous ammonia workup was shown to have a wider scope than ever reported, affording the respective 3-(amino)-methyleneindoline-2-thiones and 3-(amino)methyleneindolin-2-ones directly in reasonable yields. Thus, our aqueous ammonia workup expanded the scope of the Vilsmeier formylation and allowed for the first time a very concise two-pot synthesis of wasalexins (**17**) and several new brassilexin analogues. Finally, the antifungal activity of brassilexin (**12**), sinalexin (**13**), and analogues **24a**−**j** was determined and is reported.

Results and Discussion

The most efficient preparation of brassilexin (**12**) is a two-pot synthesis,[3] starting with a Vilsmeier formylation of readily available indoline-2-thione (**9**)[14] and an unprecedented aqueous ammonia workup, followed by quantitative oxidation of 3-(amino)methyleneindoline-2-

(3) Pedras, M. S. C.; Zaharia, I. L. *Org. Lett.* **2001**, *3*, 1213.
(4) Pedras, M. S. C.; Khan, A. Q. *J. Agric. Food Chem.* **1996**, *44*, 3403.
(5) For recent reviews on cruciferous phytoalexins see: (a) Pedras, M. S. C.; Okanga, F. I.; Zaharia, I. L.; Khan, A. Q. *Phytochemistry* **2000**, *53*, 161. (b) Pedras, M. S. C.; Jha, M.; Ahiahonu, P. W. K. *Curr. Org. Chem.* **2003**, *7*, 1635.
(6) Indolin-2-ones have been used to prepare of GABAergic agents; see, for example: Sarges, R.; Howard, H. R.; Koe, B. K.; Weissman, A. *J. Med. Chem.* **1989**, *32*, 437.
(7) Indoline-2-thiones are also useful in the preparation of tyrosine kinase inhibitors, i.e., the corresponding 3-substituted 2,2′-dithiobis-(1*H*-indoles); see, for example: Palmer, B. D.; Rewcastle, G. W.; Thompson, A. M.; Boyd, M.; Showalter, H. D. H.; Sercel, A. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1995**, *38*, 58.
(8) Pedras, M. S. C.; Okanga, F. I. *J. Agric. Food Chem.* **1999**, *47*, 1196.
(9) Pedras, M. S. C.; Montaut, S.; Suchy, M. *J. Org. Chem.* **2004**, *69*, 4471.
(10) (a) Bailey, J. A.; Mansfield, J. W., Eds. *Phytoalexins*; Blackie & Son: Glasgow, UK, 1982; p 334. (b) Brooks, C. J. W.; Watson, D. G. *Nat. Prod. Rep.* **1985**, 427.
(11) Pedras, M. S. C.; Zaharia, I. L.; Gai, Y.; Zhou, Y.; Ward, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 747.
(12) (a) Rouxel, T.; Kollmann, A.; Boulidard, L.; Mithen, R. *Planta* **1991**, *184*, 271. (b) Pedras, M. S. C.; Nycholat, C. M.; Montaut, S.; Xu, Y.; Khan, A. Q. *Phytochemistry* **2002**, *59*, 611.
(13) Pedras, M. S. C.; Chumala, P. B.; Suchy, M. *Phytochemistry* **2003**, *64*, 949.
(14) Indoline-2-thione was prepared following a general procedure for conversion of the carbonyl group to the thione group: Scheeren, J. W.; Ooms, P. H. J.; Nivard, R. J. F. *Synthesis* **1973**, 149.

TABLE 1. Synthesis of Brassilexin (12) (50 mg scale)



entry	$POCl_3$ (equiv)	formylation conditions[a] (in DMF)	% products[b] brassilexin (**12**)	% products[b] **20**
1	0.5	rt, 60 min	0	93[c]
2	1	rt, 60 min	30[c]	60
3	2	rt, 60 min	60[c]	30
4	0.5	0 °C, 7 h	20 (72)[d]	<5
5	1	0 °C, 7 h	56 (55)[d]	<5
6	2	0 °C, 7 h	80 (48)[d]	<5
7	1	40−50 °C, 25 min	54[c]	35[c]
8	2	40−50 °C, 25 min	71[c]	<5

[a] Ammonia workup at 0 °C. [b] Percentage yields are averages of at least four reactions. [c] No recovery of starting material. [d] Based on recovered starting material, values in parentheses represent % of recovered material. Similar yields were obtained using 100−200 mg of **9**.

thione (**19**)[15] to brassilexin (**12**) using I_2 in pyridine (Scheme 3). Because the overall yield of this synthesis is determined by the yield of the Vilsmeier formylation,[3] it was of interest to investigate the Vilsmeier reaction conditions used to formylate indoline-2-thione (**9**). Three procedures were used: the thione **9** dissolved in DMF (50 mg scale) was added slowly by syringe to the Vilsmeier reagent ($POCl_3$/DMF) prepared in situ, or the solid thione was added by cannula, or the Vilsmeier reagent dissolved in $CHCl_3$ was added by syringe to a DMF solution of the thione. The reaction mixture was monitored by TLC and after the thione **9** was consumed, ice-cold ammonium hydroxide (excess) was added, followed by extraction. Direct addition of the Vilsmeier reagent to the thione resulted in a lower yield of **19** than adding the thione to the Vilsmeier reagent, whereas the yields were similar whether the thione was added in solution or as a solid. When the solvent was changed from DMF to $CHCl_3$ and the formylating agent was oxalyl chloride/DMF the product yields were ca. 15% lower. Eventually, we settled on the procedure technically easier to carry out, addition of the solid thione **9** by cannula to $POCl_3$/DMF, followed by aqueous ammonia workup. Subsequently, to optimize the formylation yields, the conditions for the Vilsmeier reaction were modified as summarized in Table 1. Importantly, it was determined that the use of 0.5 equiv of $POCl_3$ (with respect to the thione **9**) led to the exclusive formation of 5*H*-thiopyrano-[2,3-*b*:6,5-*b*′]diindole (**20**) in 93% yield (Table 1, entry 1), whereas the use of 1 equiv of $POCl_3$ resulted in the formation of both **20** and brassilexin (**12**) in 70% and 30% yield, respectively (Table 1, entry 2). When the amount of $POCl_3$ was further increased (2 equiv), the yield of brassilexin (**12**) increased to 60% and that of **20** was reduced to 30% (Table 1, entry 3); however, an additional increase of $POCl_3$ did not improve the yield of brassilexin (**12**). The temperature of the Vilsmeier reaction affected differently the formation of enamine **19** (through its precursor *N*,*N*-dimethylenamine **21)** and **20**. For example, at 0 °C the rates of both reactions decreased substantially, but the formation of **20** was slower than that of **12** (Table 1, entries 4−6).[16] The best yield of **12** was obtained within a temperature range of 40−50 °C (Table 1, entries 7 and 8); reactions carried out at higher temperatures (90−100 °C) resulted in the formation of multiple side products. The reaction of indoline-2-thione (**9**) with 2 equiv of $POCl_3$ in DMF at 40−50 °C afforded the best yield of brassilexin (**12**, 71%) (Table 1, entry 8).[17] To determine if the aqueous ammonia workup was promoting the formation of undesired products, the Vilsmeier intermediate 3-(*N*,*N*-dimethylamino)methyleneindoline-2-thione (**21**) was treated with an excess of ammonia and extracted, the extract was concentrated to dryness, and the residue was subjected to I_2 oxidation to yield brassilexin (**12**) in very good yield (88%).[18] This result confirmed that the ammonia workup did not promote formation of **20** or other side products.

Next, we examined the pathway for the formation of 5*H*-thiopyrano[2,3-*b*:6,5-*b*′]diindole (**20**).[3] A solution of indoline-2-thione (**9**) and 3-(*N*,*N*-dimethylamino)methyleneindoline-2-thione (**21**) in DMF was allowed to react under acidic conditions at room temperature (HCl gas bubbled in) to yield compound **20** in 77% yield. This result together with those reported in Table 1 indicated that **20** results from condensation of the Vilsmeier adduct **21** with the thione/thiol **9**/**9a**. The proposed stepwise formation is summarized in Scheme 4. The classic Vilsmeier formylation step leading to **21** might be followed by nucleophilic attack of ene-thiol **9a**, leading to unstable intermediates $\mathbf{I_1}$ and $\mathbf{I_2}$ or equivalents. This $\mathbf{I_2}$ intermediate could rapidly undergo rearomatization with elimination of H_2S to yield the novel heteroaromatic 5*H*-thiopyrano[2,3-*b*:6,5-*b*′]diindole (**20**). When aqueous ammonia is used for workup of the Vilsmeier formylation, dimethylamine **21** is transformed into enamine **19**.[19]

After having improved the formylation−amination yield of indoline-2-thione (**9**), we examined the Vilsmeier

(15) Enamine **19** was previously prepared by reduction of 2-mercaptoindole-3-carboxaldehyde oxime using $TiCl_3$/$NaBH_3CN$, as reported in ref 8; **19** oxidizes slowly to **12** (<5%, 24 h) on standing in CH_2Cl_2.

(16) Although the results shown in Table 1 entry 6 represented an improvement in the yield of **12**, due to the amount of starting material remaining in the reaction mixture, the additional chromatographic separation required rendered this preparation less efficient.

(17) A number of undetermined products were formed including 2-chloroindole-3-carbaldeheyde (**11**) in ca. 10%.

(18) Compound **21** was synthesized using the conditions of entry 8, Table 1 followed by water workup instead of ammonia.

(19) For complete spectroscopic data of **19**, see ref 8.

SCHEME 4. Proposed Stepwise Formation of 5*H*-Thiopyrano[2,3-*b*:6,5-*b*′]diindole (20)

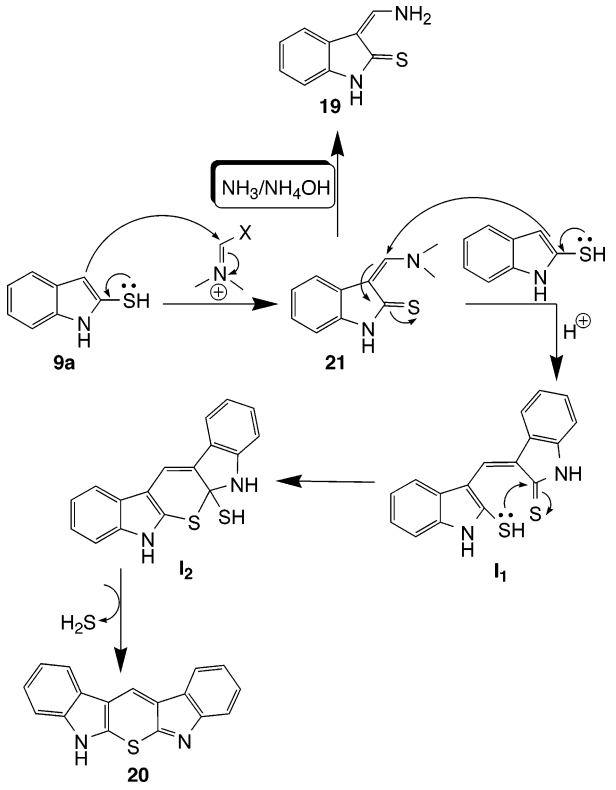
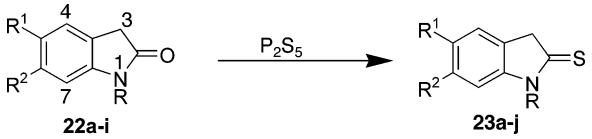


TABLE 2. Synthesis of 5- and 6-Substituted Indoline-2-thiones 23a−j



entry	starting material	product **23**	R	R^1	R^2	yield (%)
1	**22a**	**a**	H	OCH_3	H	82
2	**22b**	**b**	H	CH_3	H	93
3	**22c**	**c**	H	F	H	90
4	**22d**	**d**	H	Cl	H	95
5	**22e**	**e**	H	Br	H	78
6	**22f**	**f**	H	H	OCH_3	82
7	**22 g**	**g**	H	H	F	81
8	**22h**	**h**	H	H	Cl	92
9	**22i**	**i**	H	H	Br	74
10	**7**	**j**	CH_3	H	H	94

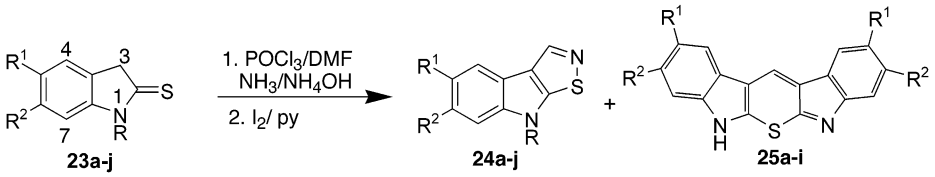
formylation of substituted indoline-2-thiones. 5-Substituted indoline-2-thiones **23a**−**e** were synthesized by thiation[14] of indolin-2-ones **22a**−**e** (Table 2, entries 1−5), which were prepared from the corresponding 5-substituted isatins using a modification of the Wolff−Kishner reduction,[20] as described in the Supporting Information. 6-Substituted indoline-2-thiones **23f**−**i** were synthesized in four steps starting from substituted α-halonitrobenzenes, using an excess of dimethylmalonate in DMF and *t*-BuOK.[21−23] Thiation of the purified indolin-2-ones **22f**−**i** was carried out using phosphorus pentasulfide[14] in THF to yield indoline-2-thiones **23f**−**i** (Table 2, entries 6−9). 1-Methylindoline-2-thione (**23j**) and 1-methoxyindoline-2-thione (**10**) were prepared by thiation of 1-methylindolin-2-one (**7**) and 1-methoxyindolin-2-one (**10**, R = OMe, Y = O).[14]

As shown in Table 3, the presence of electron-withdrawing or electron-donating substituents at C-5 or C-6 did not appear to affect noticeably the reactivity of the indoline-2-thiones toward the Vilsmeier formylation. Similar to the formylation of indoline-2-thione (**9**), the formylation of indoline-2-thiones **23a**−**j** was completed within 20−30 min at 40−50 °C (Table 3, entries 1−9). However, in the case of thiones **23j** (R = Me, $R^1 = R^2$ = H) and **10** (R = OMe, Y = O), used for synthesis of 1-methylbrassilexin (**24j**) and sinalexin (**13**), respectively, temperatures higher than 25 °C resulted in the formation of multiple undetermined products, whereas at 25 °C the formylation reactions proceeded in reasonable yields (Table 3, entries 10 and 12). The pentacyclic products **25a**−**i** were formed in every reaction except where substitution at $N - 1$ of the starting material prevented their formation (**23j** and **10**). Products **25a**−**i** were characterized through their methyl derivatives due to poor solubility of the parent compounds in NMR deuterated solvents.[23]

After successful application of the Vilsmeier formylation to indoline-2-thiones, we have examined the formylation of indolin-2-ones, useful starting materials for the synthesis of wasalexins (**17**) and analogues. Indolin-2-ones **7**,[25] **7a**,[26] and **7b**[27] (Table 4, entries 2−4) were synthesized as previously reported. Similar to the formylation of thiones **23a**−**j**, the Vilsmeier formylation of indolin-2-ones was carried out in DMF, but using only 1.2 equiv of $POCl_3$ (Table 4). After completion of the reaction, the reaction mixture was cooled to 0 °C, quenched with ice-cold aqueous ammonia, and allowed to warm to room temperature under continuous stirring to yield, after extraction, enamines **26a**−**c** in good yields. Enamine **26d** was not formed, instead the acetyl group was hydrolyzed during the ammonia workup to yield the deprotected enamine **26a**. Alternatively, the Vilsmeier reaction mixture was quenched with ice-cold H_2O and extracted to yield dimethylenamines **27a**−**d** (Table 4).[28] Next, enamines **26a**−**c** were allowed to react with NaH and CS_2 in THF, followed by treatment with MeI and workup to yield directly wasalexins (**17**) and analogue **17a** in good yields (Scheme 5). This new synthesis of wasalexins is a concise and fast two-pot preparation followed by one chromatographic separation, that affords wasalexins in 68% overall yield. This procedure represents a substantial improvement to our previous synthesis of wasalexins (**17**) from 1-methoxyindolin-2-one (**7a**), which was carried out in four steps and 54% overall yield.[13] In addition, it is particularly worthy to note that

(20) Huang, M. *J. Am. Chem. Soc.* **1949**, *71*, 3301.
(21) Goetz, F. J.; Hirsch, J. A.; Augustine, R. L. *J. Org. Chem.* **1983**, *48*, 2468.
(22) Quallich, G. J.; Morrissey, P. M. *Synthesis* **1993**, 51.
(23) Described in the Supporting Information.
(24) Compound **24a** was previously prepared using a different procedure and undisclosed yield; see: Tempete, C.; Devys, M.; Barbier, M. *Z. Naturforsch., C: J. Biosci.* **1991**, *46*, 706.
(25) Crestini, C.; Saladino, R. *Synth. Commun.* **1994**, *24*, 2835.
(26) Thompson, M. E. *J. Org. Chem.* **1984**, *49*, 1700.
(27) Robertson, D. W.; Krushinski, J. H.; Kau, D. *J. Labelled Compd. Radiopharm.* **1986**, *23*, 343.
(28) Compound **27d** was prepared previously using Vilsmeier conditions in 30% yield; see: Andreani, A.; Bonazzi, D.; Rambaldi, M.; Mungiovino, G.; Greci, L. *Farmaco* **1978**, *33*, 781.

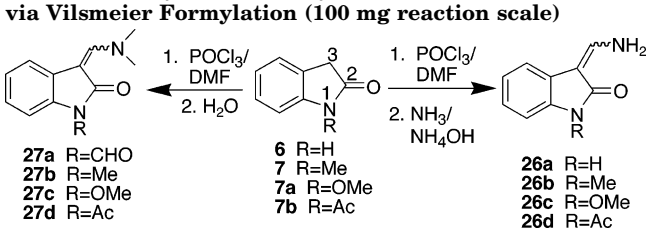
TABLE 3. Syntheses of Brassilexin Analogues via Vilsmeier Formylation (50 mg scale)[a]



entry	starting material	main product	R	R^1	R^2	yield (%)
1	**23a**	**24a**[24]	H	OCH_3	H	67
2	**23b**	**24b**	H	CH_3	H	52
3	**23c**	**24c**	H	F	H	64
4	**23d**	**24d**	H	Cl	H	54
5	**23e**	**24e**	H	Br	H	40
6	**23f**	**24f**	H	H	OCH_3	47
7	**23g**	**24g**	H	H	F	68
8	**23h**	**24h**	H	H	Cl	58
9	**23i**	**24i**	H	H	Br	45
10[a]	**23j**	**24j**[b]	CH_3	H	H	65
11[b]	**9**	**12**[c]	H	H	H	71
12[a]	**10**	**13**[b]	OCH_3	H	H	67

[a] Reactions were carried out at 40 °C, except for **23i** and **10** which were carried out at 25° C. [b] Minor product **25** is not formed. [c] Minor product **20** is formed.

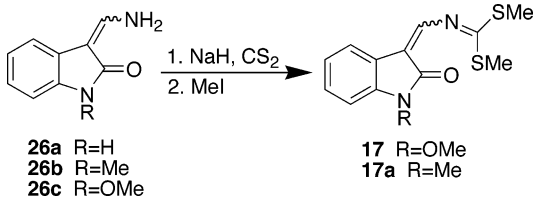
TABLE 4. Synthesis of 3-(Amino)methyleneindolin-2-ones 26a–c and 3-(*N,N*-Dimethylamino)methyleneindolin-2-ones 27a–d via Vilsmeier Formylation (100 mg reaction scale)



entry	starting material	formylation conditions[a]	product[b] of H_2O workup at 0 °C (%)	product[b] of NH_3/NH_4OH workup at 0 °C (%)
1	**6**	40 °C, 30 min	**27a** (88)	**26a** (82)
2	**7**	40 °C, 60 min	**27b** (81)	**26b** (83)
3	**7a**	40 °C, 60 min	**27c** (86)	**26c** (86)
4	**7b**	40 °C, 120 min	**27d** (85)	only **26a** (80)

[a] 1.2 equiv of $POCl_3$ in DMF. [b] Mixture of *E*/*Z* isomers.

SCHEME 5. Synthesis of Wasalexins (17) and Analogue 17a



imines **26a–c** were obtained directly in a one-pot synthesis for the first time.[29] Interestingly, formylation of the *N* − 1 of **6** occurred under mild Vilsmeier conditions to yield **27a**, whereas ammonia workup yielded the desired enamine **26a** in good yield.

Antifungal Activity. To establish the antifungal activity of the new phytoalexin analogues, bioassays were carried out against *Phoma lingam* (Tode ex Fr.) Desm. (perfect stage *Leptosphaeria maculans* (Desm.) Ces. et de Not.), the so-called blackleg fungus, one of the major fungal pathogens of cruciferous crops.[30] As shown in Table 5, the antifungal activity of the naturally occurring compounds **12** and **13** against *P. lingam*/*L. maculans* is quite similar. In general, the results suggest that the position of the substituent has stronger influence on the activity of the analogues than the nature of the substituent itself. For example, the 6-F, 6-Cl, and 6-Br derivatives display stronger activity than the 5-substituted compounds, except for the 5- and 6-OMe derivatives which appear to display similar antifungal activity.

Conclusion

The unprecedented ammonia workup of the Vilsmeier formylation reaction is crucial for the preparation of primary imine/enamines of both methyleneindoline-2-thiones and methyleneindolin-2-ones, because these products cannot be obtained directly from reactions of the corresponding aldehydes with ammonia.[31] Several aminomethyleneindoline-2-thiones were prepared for the first time using this method and were used to prepare several new brassilexin analogues (**24b–j**). Furthermore, the formylation–amination of indolin-2-ones allowed the most concise synthesis of wasalexins (**17**). This process could be used to prepare 5- or 6-substituted wasalexins. Importantly, these procedures will allow more efficient syntheses of isotopically labeled phytoalexins and intermediates that have immediate application in the investigation of plant and fungal metabolic pathways.

The antifungal activity of brassilexin (**12**), sinalexin (**13**), and analogues **24a–j** toward the blackleg fungus (*L. maculans*/*P. lingam*) was determined. Because this fungus is able to detoxify brassilexin (**12**) via an efficient but undetermined pathway,[8] compounds **24a–j** will be valuable tools to probe this detoxification pathway as well as the selectivity of enzyme(s) involved in it.

(29) Enamines **26a–c** exist in CH_3CN solution as a mixture of *E*/*Z* isomers (spectroscopic data in the Supporting Information).

(30) For recent work on this pathogen see, for example: Pedras, M. S. C.; Chumala, P. B. *Org. Lett.* **2004**,

TABLE 5. Antifungal Activity[a] of Phytoalexins and Analogues against *Phoma lingam* (perfect stage = *Leptosphaeria maculans*) (5 days incubation)

compd	concn (M)	inhibition (%) ± standard deviation[a]
12	5×10^{-4}	c.i.[b]
	2×10^{-4}	62 ± 1
	1×10^{-4}	8 ± 1
13	5×10^{-4}	c.i.
	2×10^{-4}	69 ± 4
	1×10^{-4}	24 ± 7
24a	5×10^{-4}	92 ± 4
	2×10^{-4}	40 ± 4
	1×10^{-4}	14 ± 1
24b	5×10^{-4}	c.i.
	2×10^{-4}	c.i.
	1×10^{-4}	70 ± 7
24c	5×10^{-4}	98 ± 4
	2×10^{-4}	32 ± 9
	1×10^{-4}	24 ± 19
24d	5×10^{-4}	96 ± 5
	2×10^{-4}	66 ± 7
	1×10^{-4}	n.i.[c]
24e	5×10^{-4}	72 ± 10
	2×10^{-4}	66 ± 12
	1×10^{-4}	60 ± 10
24f	5×10^{-4}	98 ± 4
	2×10^{-4}	25 ± 3
	1×10^{-4}	n.i.
24g	5×10^{-4}	c.i.
	2×10^{-4}	c.i.
	1×10^{-4}	64 ± 1
24h	5×10^{-4}	c.i.
	2×10^{-4}	c.i.
	1×10^{-4}	69 ± 6
24i	5×10^{-4}	c.i.
	2×10^{-4}	c.i.
	1×10^{-4}	c.i.
24j	5×10^{-4}	c.i.
	2×10^{-4}	72 ± 1
	1×10^{-4}	38 ± 1

[a] Percent of inhibition = 100 − [(growth on medium containing compound/growth on control medium) × 100)] ± standard deviation. [b] c.i. = complete inhibition. [c] n.i. = no inhibition (growth on control medium and on medium containing compound is similar).

Experimental Section

Antifungal Bioassays. Bioassays with *L. maculans*/*P. lingam* (isolate BJ-125) were performed using potato dextrose agar as previously described.[32]

Indoline-2-thione (9).[14] A mixture of 2-oxindole (500 mg, 3.76 mmol) and P_2S_5 (1.0 g, 2.3 mmol) in THF (25 mL) was allowed to stir for 10 min at room temperature. Sodium bicarbonate (631 mg, 7.5 mmol) was added portionwise to the above mixture and the reaction mixture was allowed to stir for 4 h at room temperature. The reaction mixture was filtered (gravity) and the excess of THF was removed under reduced pressure. Ice-cold water (50 mL) was added to the residue with vigorous stirring to afford a light yellow precipitate that was filtered and air-dried; yield 516 mg (92%); mp 144−147 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.68 (br s, 1 H, D_2O exchangeable), 7.28 (m, 2 H), 7.14 (dd, J = 7.5, 7.5 Hz, 1 H), 7.03 (d, J = 8 Hz, 1H), 4.09 (s, 2 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 203.7, 144.27, 130.3, 128.0, 124.2, 124.1, 110.0, 49.0. IR data were identical with previously reported data.[33] HRMS *m/z* measured 149.0298 (149.0299 calculated for C_8H_7NS).

Brassilexin (12). Indoline-2-thione (**9**, 50 mg, 0.33 mmol) was added to a mixture of freshly distilled $POCl_3$ (64 μL, 0.70 mmol) in DMF (350 μL) at 45 °C and the temperature of the reaction mixture was kept at 45 °C. After 20 min, the reaction mixture was cooled to 0 °C on an ice bath, and aq NH_4OH (29%, 8 mL) was added dropwise with constant stirring. The resulting solution was allowed to warm to room temperature and extracted with Et_2O, and the organic extracts were combined, dried over Na_2SO_4, and concentrated to dryness. The crude product was dissolved in pyridine (1 mL) and I_2 (42 mg, 0.33 mmol) was added to the reaction mixture. After being stirred for 60 min at room temperature, the reaction mixture was acidified (1.5 M H_2SO_4, 10 mL), the resulting solution was extracted with Et_2O, and the organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness. The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc− hexane, 20:80) to afford 42 mg of **12** (71%); mp 140−142 °C.

Brassilexin (12) from 3-(*N,N*-Dimethylamino)methyleneindoline-2-thione (21). Indoline-2-thione **21** (20 mg, 0.14 mmol) was dissolved in DMF (300 μL) and aq ammonium hydroxide (2 mL) was added. After being stirred for 10 min, the mixture was diluted with H_2O (10 mL) and extracted with Et_2O, and the organic extracts were combined, dried over Na_2SO_4, and concentrated to dryness. I_2 (34 mg, 0.14 mmol) was added to the crude product dissolved in pyridine (1 mL) and the reaction mixture was stirred for 60 min at room temperature. After acidification (H_2SO_4,10 mL) the resulting solution was extracted with Et_2O, and the organic extracts were combined, dried over Na_2SO_4, and concentrated to dryness. The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 15 mg of **12** (88%); mp 140−142 °C.

Sinalexin (13). Preparation was the same as reported for brassilexin (**12**) but the temperature of the reaction was kept at room temperature. The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 38 mg of **13** (67%); mp 36−37 °C.

3-(*N,N*-Dimethylamino)methyleneindoline-2-thione (21). Indoline-2-thione (**9**, 200 mg, 1.34 mmol) was added to a mixture of freshly distilled $POCl_3$ (260 μL, 2.8 mmol) in DMF (1.7 mL) at 45 °C, and the reaction mixture was kept at 45 °C. After 90 min the reaction mixture was cooled to 0 °C on an ice bath and diluted withice-cold water (20 mL), then the precipitate formed was filtered off. The filtrate was extracted with Et_2O, and the organic extracts were combined, dried over Na_2SO_4, and concentrated to dryness. The crude product was subjected to flash column chromatography (silica gel, CH_2Cl_2) to yield 168 mg of **21** (60%). ^{1}H NMR (500 MHz, CD_3CN) δ 9.8 (br s, 1 H), 8.41 (s, 1 H), 7.49 (d, J = 7.5 Hz, 1 H), 7.08 (d, J = 3.5 Hz, 1H), 7.04 (m, 2 H), 3.46 (s, 6 H). ^{13}C NMR (500 MHz, CD_3CN, 50 °C) δ 186.2 (br s), 157.5, 139.8, 125.5, 123.5, 121.6, 120.3, 109.6, 108.4, 45.1 (br s, 2 CH_3). FTIR ν_{max} (KBr): 3089 (br), 2932, 1626, 1447, 1312, 1184, 745 cm^{-1}. HRMS *m/z* measured 204.0171 (204.0721 calculated for $C_{11}H_{12}N_2S$).

5-Methoxybrassilexin (24a). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 39 mg of **24a** (67%); mp 169−172 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.71 (br s, 1 H, D_2O exchangeable), 8.67 (s, 1 H), 7.48 (m, 2 H), 6.97 (dd, J = 9, 2.5 Hz, 1H), 3.87 (s, 3 H). ^{13}C NMR (500 MHz, CD_3CN) δ 159.7, 154.9, 147.7, 139.3, 127.6, 120.9, 113.1, 112.9, 103.2, 55.7. FTIR ν_{max} (KBr) 3150, 1623, 1468, 1212, 1068, 789 cm^{-1}. HRMS *m/z* measured 204.0357 (204.0356 calculated for $C_{10}H_8N_2OS$). EIMS *m/z* (% relative abundance) 204 (M^+, 100), 189 (59), 161 (23), 134 (9).

(31) The reaction of ammonia with aldehydes leads to spontaneous polymerization of the resulting imines. (a) Smith, M. B.; March, J. *Advanced Organic Chemistry*, 5th ed.; J. Wiley & Sons: New York, 2001; p 1186. (b) Hull, W. E.; Sykes, B. D.; Babior, B. M. *J. Org. Chem.* **1973**, *38*, 2931.

(32) Pedras, M. S. C.; Montaut, S. *Bioorg. Med. Chem.* **2003**, *11*, 3115.

(33) Rewcastle, G. W.; Janosik, T.; Bergman, J. *Tetrahedron* **2001**, *57*, 7185.

5-Methylbrassilexin (24b). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 30 mg of **24b** (52%); mp 171−174 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.76 (br s, 1 H, D_2O exchangeable), 8.66 (s, 1 H), 7.71 (s, 1 H), 7.44 (d, J = 8.5 Hz, 1H), 7.16 (d, J = 8 Hz, 1 H), 2.47 (s, 3 H). ^{13}C NMR (500 MHz, CD_3CN) δ 159.5, 147.7, 142.9, 130.3, 127.4, 125.3, 120.5, 120.0, 112.1, 20.8. FTIR ν_{max} (KBr) 3120, 3051, 2892, 2276, 1513, 1473, 1244, 788 cm^{-1}. HRMS *m/z* measured 188.0408 (188.0403 calculated for $C_{10}H_8N_2S$). EIMS *m/z* (% relative abundance) 188 (M^+, 100), 187 (35), 155 (34).

5-Fluorobrassilexin (24c). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 38 mg of **24c** (64%); mp 198−200 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.97 (br s, 1 H, D_2O exchangeable), 8.68 (s, 1 H), 7.65 (dd, J = 9.5, 2.5 Hz, 1 H), 7.55 (dd, J = 9, 4.5 Hz, 1 H), 7.12 (ddd, J = 9.5, 9, 2.5 Hz, 1 H). ^{13}C NMR (500 MHz, CD_3CN) δ 160.7, 158.2 (d, J_{C-F} = 233 Hz), 147.8, 141.0, 127.3 (d, J_{C-F} = 6), 126.7 (d, J_{C-F} = 10 Hz), 113.4 (d, J_{C-F} = 10 Hz), 111.6 (d, J_{C-F} = 27 Hz), 105.8 (d, J_{C-F} = 25 Hz). FTIR ν_{max} (KBr) 3143, 3075, 2968, 2894, 1588, 1473, 1364, 1182, 801 cm^{-1}. HRMS *m/z* measured 192.0157 (192.0150 calculated for $C_9H_5N_2SF$). EIMS *m/z* (% relative abundance) 192 (M^+, 100), 160 (16), 149 (24), 137 (13).

5-Chlorobrassilexin (24d). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 35 mg of **24d** (60%); mp 226−227 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 10.0 (br s, 1 H, D_2O exchangeable), 8.7 (s, 1 H), 7.95 (d, J = 2 Hz, 1 H), 7.59 (d, J = 9 Hz, 1 H), 7.33 (dd, J = 8.5, 1.5 Hz, 1 H). ^{13}C NMR (500 MHz, CD_3CN) δ 160.5, 147.8, 142.9, 126.8, 125.8, 123.9 121.3, 119.8, 113.7. FTIR ν_{max} (KBr) 3130, 3047, 2953, 2887, 1505, 1462, 1234, 786 cm^{-1}. HRMS *m/z* measured 207.9861 (207.9861 calculated for $C_9H_5N_2SCl$). EIMS *m/z* (% relative abundance) 210 (34), 208 (M^+, 100), 146 (10), 103 (10).

5-Bromobrassilexin (24e). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 23 mg of **24e** (40%); mp 222−223 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.98 (br s, 1 H, D_2O exchangeable), 8.69 (s, 1 H), 8.1 (s, 1 H), 7.51 (d, J = 8.5 Hz, 1 H), 7.45 (d, J = 8.5 Hz, 1 H). ^{13}C NMR (500 MHz, CD_3CN) δ 160.3, 147.9, 143.2, 126.6, 124.9, 123.0, 121.9, 114.9, 113.2. FTIR ν_{max} (KBr) 3125, 2946, 2886, 1506, 1459, 1233, 786 cm^{-1}. HRMS *m/z* measured 253.9336 (253.9330 calculated for $C_9H_5N_2SBr$). EIMS *m/z* (% relative abundance) 254 (M^+, 100), 252 (98), 173 (74), 146 (18).

6-Methoxybrassilexin (24f). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 27 mg of **24f** (47%); mp 174−176 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.75 (br s, 1 H, D_2O exchangeable), 8.62 (s, 1 H), 7.79 (d, J = 8.5 Hz, 1 H), 7.12 (d, J = 2.5 Hz, 1H), 6.87 (dd, J = 8.5, 2.5 Hz, 1 H), 3.87 (s, 3 H). ^{13}C NMR (500 MHz, CD_3CN) δ 158.7, 157.7, 147.2, 145.7, 127.5, 120.7, 114.3, 109.7, 96.4, 55.5. FTIR ν_{max} (KBr) 3130, 2993, 2909, 1627, 1589, 1508, 1368, 1138, 961 cm^{-1}. HRMS *m/z* measured 204.035735 (204.035439 calculated for $C_{10}H_8N_2OS$). EIMS *m/z* (% relative abundance) 204 (M^+, 100), 189 (91), 161 (15), 100 (9).

6-Fluorobrassilexin (24g). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 39 mg of **24g** (68%); mp 168−170 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.93 (br s, 1 H, D_2O exchangeable), 8.67 (s, 1 H), 7.89 (dd, J = 8.5, 8.5 Hz, 1 H), 7.35 (dd, J = 10, 2.5 Hz, 1 H), 7.03 (ddd, J = 9.5, 9, 2.5 Hz, 1 H). ^{13}C NMR (500 MHz, CD_3CN) δ 160.5 (d, J_{C-F} = 235 Hz), 159.9, 147.5, 144.8 (d, J_{C-F} = 12.5 Hz), 127.2, 121.2 (d, J_{C-F} = 10 Hz), 117.0, 108.6 (d, J_{C-F} = 25 Hz), 99.1(d, J_{C-F} = 26 Hz). FTIR ν_{max} (KBr) 3130, 2993, 2909, 1627, 1589, 1508, 1368, 1138, 961 cm^{-1}. HRMS *m/z* measured 192.0157 (192.0158 calculated for $C_9H_5N_2SF$). EIMS *m/z* (% relative abundance) 192 (M^+, 100), 164 (14), 160 (16), 137 (16).

6-Chlorobrassilexin (24h). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 34 mg of **24h** (58%); mp 184−186 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.93 (br s, 1 H, D_2O exchangeable), 8.68 (s, 1 H), 7.86 (d, J = 8.5 Hz, 1 H), 7.59 (d, J = 1.5 Hz, 1 H), 7.22 (dd, J = 8.5, 1.5 Hz, 1 H). ^{13}C NMR (500 MHz, CD_3CN) δ 160.1, 147.8, 144.8, 129.1, 127.1, 121.2, 121.0, 119.0, 112.3. FTIR ν_{max} (KBr) 3107 (br), 2964, 2886, 1564, 1501, 1458, 1239, 1056, 851, 795 cm^{-1}. HRMS *m/z* measured 207.9861 (207.9856 calculated for $C_9H_5N_2SCl$). EIMS *m/z* (% relative abundance) 210 (34), 208 (M^+, 100), 176, (45), 153 (12), 103 (8).

6-Bromobrassilexin (24i). Preparation was the same as reported for brassilexin (**12**). The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 25 mg of **24i** (45%); mp 182−185 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 9.93 (br s, 1H, D_2O exchangeable), 8.7 (s, 1 H), 7.84 (d, J = 8.5, 1H), 7.64 (d, J = 1.5, 1H), 7.38 (dd, J = 8.5, 1.5, 1H). ^{13}C NMR (500 MHz, CD_3CN) δ 160.1, 147.9, 145.2, 127.2, 123.8, 121.6, 119.4, 116.6, 115.3. FTIR ν_{max} (KBr) 3101 (br), 1641, 1570, 1502, 1423, 1367, 1237, 846 cm^{-1}. HRMS *m/z* measured 253.9336 (253.9336 calculated for $C_9H_5N_2SBr$). EIMS *m/z* (% relative abundance) 254 (M^+, 100), 252 (98), 173 (81), 146 (14).

1-Methylbrassilexin (24j). Preparation was the same as reported for brassilexin (**12**) but the temperature of the reaction was kept at room temperature. The crude reaction mixture was subjected to flash column chromatography (silica gel, EtOAc−hexane, 20:80) to afford 37 mg of **13** (65%); mp 67−69 °C. ^{1}H NMR (500 MHz, CD_3CN) δ 8.71 (s, 1 H), 7.93 (d, J = 8 Hz, 1 H), 7.48 (d, J = 8 Hz, 1 H), 7.4 (dd, J = 8, 7.5 Hz, 1H), 7.26 (dd, J = 7.5, 7.5 Hz, 1 H), 3.87 (s, 3 H). ^{13}C NMR (500 MHz, CD_3CN) δ 162.1, 148.3, 145.1, 125.9, 123.9, 120.8, 120.4, 120.3, 110.3, 33.1. FTIR ν_{max} (KBr) 1490, 1464, 1319, 1261, 912, 743 cm^{-1}. HRMS *m/z* measured 188.04082 (188.040773 calculated for $C_{10}H_8N_2S$). EIMS *m/z* (% relative abundance) 188 (M^+, 100), 155 (15), 146 (11).

Acknowledgment. Financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC Discovery and Strategic Grants to M.S.C.P) and the University of Saskatchewan (teaching assistantship to M.J.) is gratefully acknowledged.

Note Added after ASAP Publication. Table 1 contained coding errors in the version published ASAP February 3, 2005, and a Review-Only Supporting Information file was posted. The corrected version was published ASAP February 4, 2005.

Supporting Information Available: General experimental and characterization data for compounds **23a**−**j**, **25a**−**i**, and **27a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0479866