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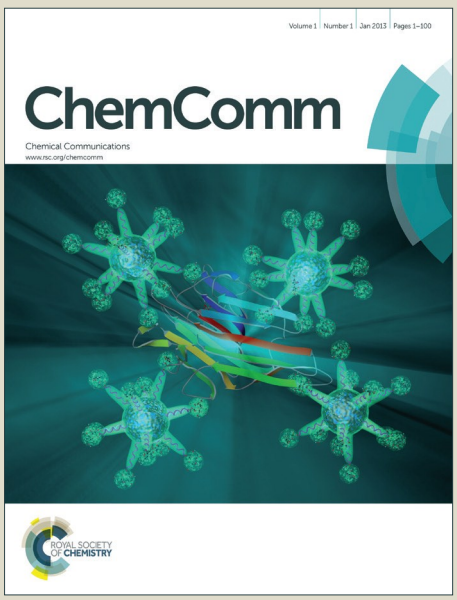

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Divergent reactivity of an indole glucosinolate yields Lossen or Neber rearrangement products: the phytoalexin rapalexin A or a unique β-D-glucopyranose fused heterocycle

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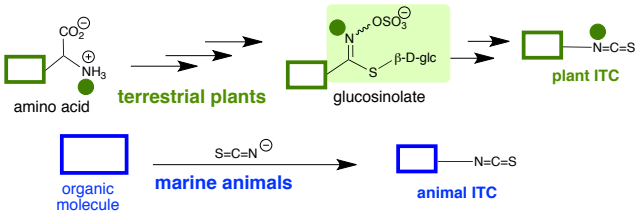
Transformation of 1-*t*-Boc-4-methoxyindole-3-glucosinolate in acidic conditions yielded the potent phytoalexin rapalexin A, providing its first biomimetic synthesis via Lossen type rearrangement, while the novel 1-thioimidocarbonyl-β-D-glucopyranose heterocyclic system was obtained in basic conditions via Neber type rearrangement.

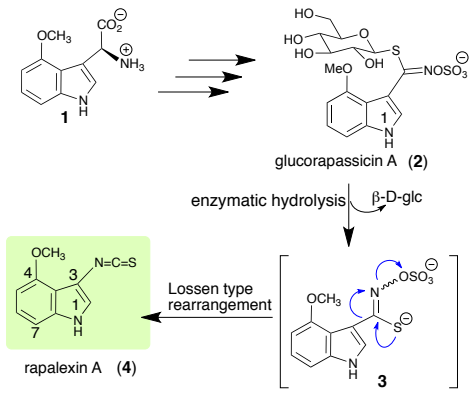
Glucosinolates are plant metabolites that are precursors of isothiocyanates (–N=C=S, ITC) and other natural products produced in crucifer species.[1] Natural products containing the ITC group are both ubiquitous and structurally diverse. Notably, while the vast majority of ITCs produced in terrestrial plants are formed in complex pathways, generating glucosinolates from amino acids and culminating with Lossen type rearrangements,[1] marine animals use inorganic ITC to add to an organic skeleton (Scheme 1).[2] ITCs have important defensive roles and are necessary for the survival of plants and animals in highly competitive environments.[1,2] Along with these ecological functions, toxicological and epidemiological work suggests that plant ITCs have chemopreventive roles against certain types of cancer in mammalians.[3,4]

Rapalexin A (**4**) is a potent indole-ITC phytoalexin isolated from crucifers cultivated worldwide for oils (e.g. canola and rapeseed) and other nutritional products (e.g. broccoli, cabbage, cauliflower, turnip).[5] Phytoalexins are natural products produced *de novo* by plants in response to stress caused by biotic or abiotic factors. In many circumstances, these natural defenses are crucial in plant resistance to diseases caused by microbial pathogens.[6] For this reason, their occurrence and pathways continue to generate enormous interest and to stimulate work to discover ecologically sensible approaches that produce crops able to withstand microbial threats in pesticide free environments.[6,7]



Scheme 1 Biosynthetic pathways of ITCs in terrestrial plants (N= ● is derived from the amino acid) and in marine animals.

By analogy to other ITCs produced in crucifer species (Scheme 1), the biosynthetic pathway of rapalexin A (**4**) was proposed to start with the amino acid 4-methoxyindolylglycine (**1**), followed by formation of the glucosinolate glucorapassicin A (**2**), subsequent hydrolysis of the glucosyl residue and a spontaneous Lossen type rearrangement (Scheme 2). Recent biosynthetic studies with isotopically labeled precursors verified this proposal, although glucorapassicin A (**2**) was not included because its chemical synthesis was unsuccessful.[8] Herein we report further work toward the synthesis of glucorapassicin A (**2**) leading to the first biomimetic route to rapalexin A (**4**) via Lossen rearrangement, and to a unique bicyclic glucose fused product (**14**) formed via Neber rearrangement. Details of the chemistry of these intriguing transformations are disclosed.



Scheme 2 Biosynthetic pathway of rapalexin A (**4**) in crucifers.

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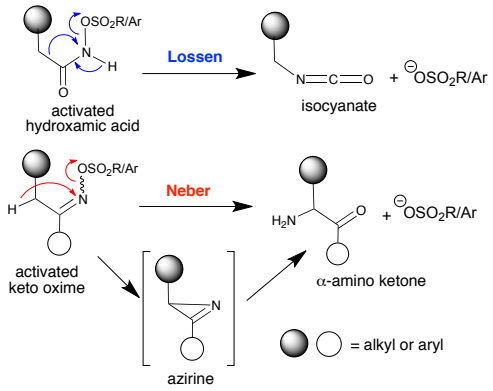
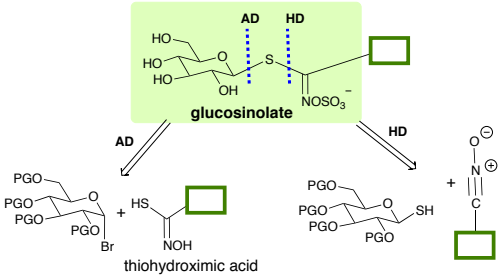
The classical Lossen rearrangement involves the transformation of *O*-activated hydroxamic acids,[9] whereas the Neber rearrangement occurs with *O*-activated ketoximes.[10,11] Typically, *O*-activated substrates carry Ar/R-sulfonyl or Ar/R-acyl groups (Scheme 3); variations of these reactions discovered over several decades afford Lossen type and Neber type rearrangement products.[9,10]

The syntheses of aryl and indolyl glucosinolates have been achieved using two approaches, the so-called "anomeric disconnection" (**AD**) and the "hydroximate disconnection" (**HD**) (Scheme 4).[12] Using the hydroximate disconnection,[12] previous attempts to synthesize glucorapassicin A (**2**) afforded 1-*t*-Boc-glucorapassicin A (**10**),[8] which decomposed on standing in either aqueous or organic solvents over a period of 24 h. For this reason, in this work another protecting group ($MeSO_2$) was used in further attempts to synthesize glucorapassicin A (**2**). Protection of 4-methoxyindole-3-carboxaldehyde (**5**) followed by oximation and chlorination using *N*-chlorosuccinimide[13] afforded the expected hydroximoyl chloride[12] (chloro oxime or *N*-hydroxyimidoyl chloride), which upon coupling with β-D-thioglucopyranose tetraacetate afforded thiohydroximate **7** in excellent yield (Scheme 5).[14] Sulfonation of **7** using Py–SO_3 complex[15] yielded sulfonic acid **8**, which was transformed to 1-$MeSO_2$-glucorapassicin A (**9**) under basic conditions, in 48% overall yield ($C_{17}H_{21}N_2O_{12}S_3K$, calc. 541.0262, obtained 541.0258). 1-*t*-Boc-glucorapassicin A (**10**) ($C_{21}H_{27}N_2O_{12}S_2K$, calc. 563.1005, obtained 563.1011) was synthesized similarly (ESI).

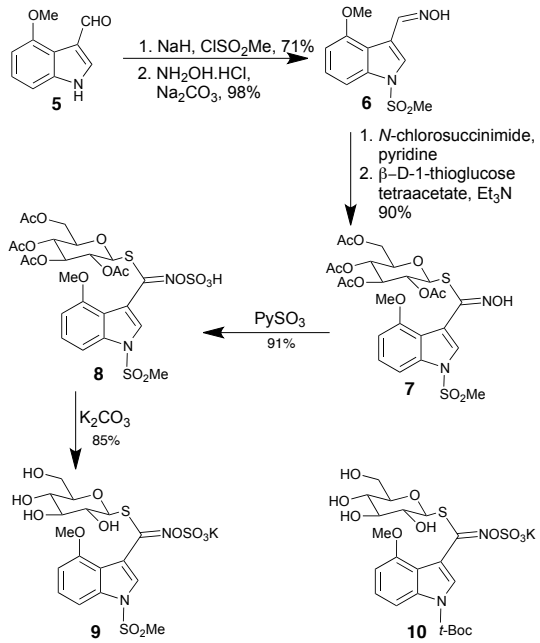
Attempts to deprotect 1-$MeSO_2$-glucorapassicin A (**9**) and 1-*t*-Boc-glucorapassicin A (**10**) under acidic conditions afforded



Scheme 3 Classical chemical reactions known as Lossen and Neber rearrangements.



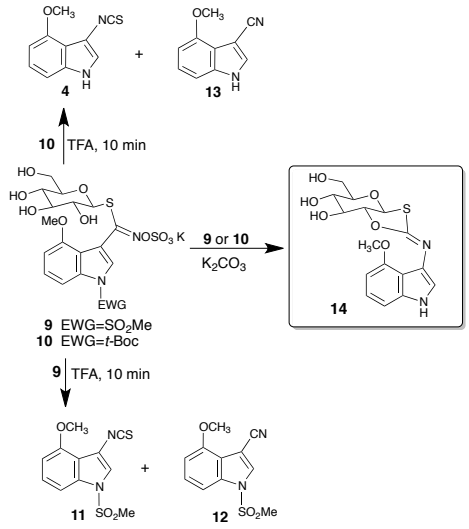
Scheme 4 General synthetic approaches to glucosinolates: **AD**, anomeric disconnection and **HD**, hydroximate disconnection;[12] PG=protecting group.



Scheme 5 Synthesis of 1-$MeSO_2$-glucorapassicin A (**9**).

four products: 1-$MeSO_2$-rapalexin A (**11**) and 1-$MeSO_2$-4-methoxyindole carbonitrile (**12**) from 1-$MeSO_2$-glucorapassicin A (**9**), and rapalexin A (**4**) and 4-methoxyindole carbonitrile (**13**) from 1-*t*-Boc-glucorapassicin A (**10**) (spectroscopic data in ESI). By contrast, deprotection of 1-$MeSO_2$-glucorapassicin A (**9**) or 1-*t*-Boc-glucorapassicin A (**10**) under basic conditions afforded consistently the major product X (**14**) (Scheme 6). That is, to our disappointment, the desired product glucorapassicin A (**2**) ($C_{16}H_{19}N_2O_{10}S_2$) was not obtained. Remarkably, formation of rapalexin A (**4**) from 1-*t*-Boc-glucorapassicin A (**10**) represents the first biomimetic synthesis of **4**, which points to a Lossen type rearrangement catalyzed by TFA.

The HRMS spectral data of product X ($C_{16}H_{18}N_2O_6S$, calc. 366.0886, obtained 366.0877) indicated the loss of both



Scheme 6 Chemical transformations of 1-EWG-glucorapassicins A **9** and **10** in acidic and basic media (EWG = electron withdrawing group).

methanesulfonyl and sulfate groups (CH_3SO_2 + SO_4K) from 1-MeSO_2-glucorapassicin A (**9**) ($C_{17}H_{21}N_2O_{12}S_3$), and *t*-Boc and sulfate groups (Me_3CCO_2 + SO_4K) from 1-*t*-Boc-glucorapassicin A (**10**) ($C_{21}H_{27}N_2O_{12}S_2$). The ^{1}H and ^{13}C NMR spectroscopic data of compound X (**14**) indicated that the spin systems of the β-D-glucopyranosyl and of the indolyl moieties were intact (^{1}H NMR obtained in CD_3OD). Methylation of product X (MeI/NaH) was carried out to establish the number of free hydroxyl substituents present in X (**14**).

MS and NMR spectroscopic data of the methylated derivative **15** were consistent with the presence of three methoxy and one *N*-methyl groups, in addition to the expected methoxy group at C-4 of indole. These results indicated that one of the hydroxyl groups of glucose was not methylated and thus it was likely attached to another atom. Based on the molecular formula of X ($C_{16}H_{18}N_2O_6S$, nine degrees of unsaturation) and comparison of its NMR, HMBC and HMQC spectroscopic data with those of 1-MeSO_2-glucorapassicin A (**9**), either chemical structures A or B appeared likely (Fig. 1). The final proof was obtained by X-ray crystallographic analysis of a single crystal of tetramethyl-X (**15**), establishing the chemical structure of X as A (Fig. 2).

Further examination of the biomimetic synthesis of rapalexin A (Scheme 6) suggested that an efficient route might be achieved if the thioglucopyranose tetraacetate were to be substituted for a more economical sulfur donor containing a leaving group. Although sulfenylation of hydroximoyl chlorides has been reported using diverse sulfides, these sulfides do not possess a reasonable leaving group, except for thioglucopyranose.[16] For this reason, HS–Si(*i*-Pr)$_3$ was considered, though it did not appear to have been applied to the sulfenylation of hydroximoyl chlorides. In a first attempt, addition of HS–Si(*i*-Pr)$_3$ to a solution of hydroximoyl chloride **17** in DCM/Et_3N and reaction monitoring by TLC indicated complete consumption of the starting material in 30 min. HRMS and ^{1}H NMR data of the reaction mixture indicated a product containing two 1-*t*-Boc-4-methoxyindolyl moieties and one Si(*i*-Pr)$_3$ group. Further modifications of the reaction conditions and isolation of the intermediate product suggested it contained bis-indolyl moieties connected by an *O*-silylated thiohydroximoyl anhydride (–(HON=)C–S–C(=NO–Si(*i*-Pr)$_3$)–). Much to our delight, treatment of this product with a solution of TFA (20-30% in DCM) yielded a mixture that, upon standing in DCM/Et_3N for 60 min, yielded rapalexin A (**4**) and 4-methoxyindole-3-carboxylic acid (**21**) (ca. 1:1). Varying the reaction temperature or the concentration of HS–Si(*i*-Pr)$_3$ (1 – 5 eq) did not affect product yields. Eventually, a one-pot synthesis of rapalexin A from oxime **16** was carried out in ca. 30% yield (Scheme 7).

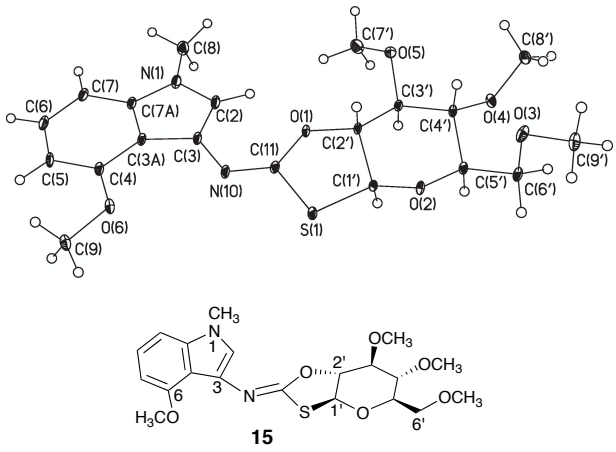


Fig. 2 Single crystal X-ray structure of compound tetramethyl-X (**15**) with thermal ellipsoids at 30% of the probability level and corresponding chemical drawing.

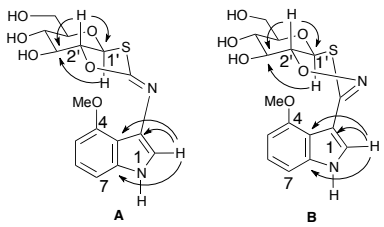
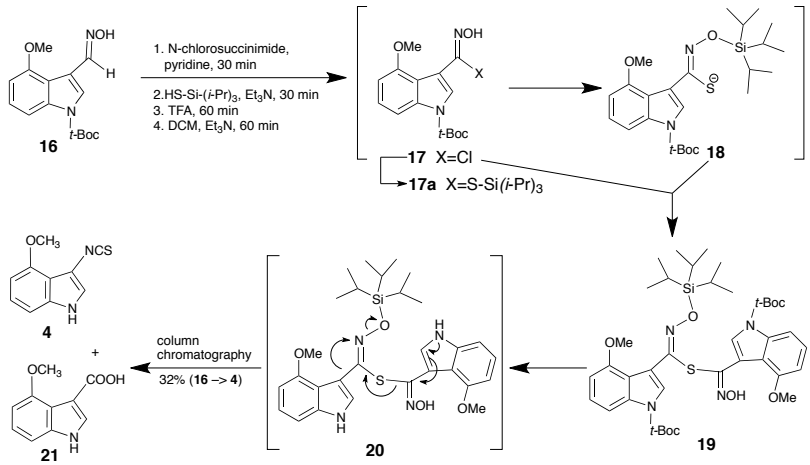
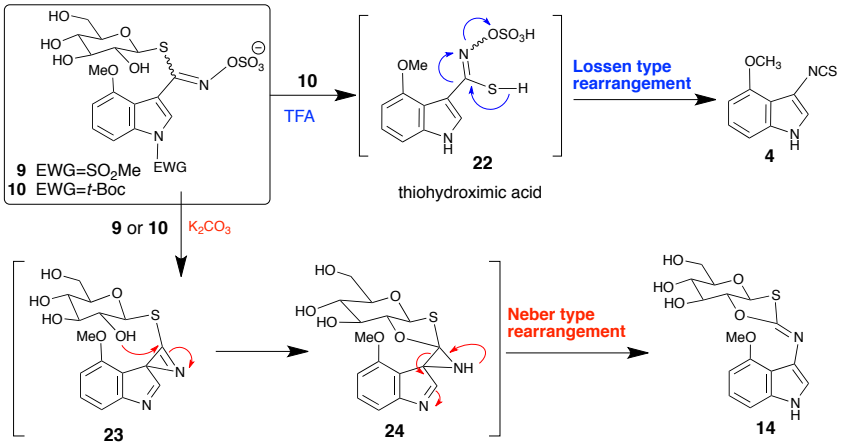


Fig. 1 Possible chemical structures of compound X (**14**) ($C_{16}H_{18}N_2O_6S$) and selected HMBC correlations.



Scheme 7 One-pot synthesis of rapalexin A (**4**) (structures in brackets are proposed intermediates).



Scheme 8 Proposed intermediates involved in formation of rapalexin A (**4**) and *N*-(4-methoxy-3-indolyl)-1-thioimidocarbonyl-β-D-glucopyranose **(14)** through Lossen and Neber type rearrangements, respectively.

The formation of product **19** is likely due to an intramolecular [1,4]-*S*- to *O*-silyl migration in the first formed reaction intermediate **17a**, followed by nucleophilic attack of the resulting sulfide **18** on the hydroximoyl carbon of **17**. This silyl migration activates the thiohydroximate, a prerequisite for the Lossen type rearrangement to occur, which is further encouraged by the *N*-deprotection of the indolyl moiety (Scheme 7). Previously, [1,4]-*S*- to O-silyl migrations were reported to proceed intramolecularly and transformed esters into ketones using organolithium reagents.[17]

As summarized in Scheme 8, hydrolysis of 1-*t*-Boc-glucorapassicin A (**10**) in acidic conditions is likely to yield the unstable intermediate thiohydroximic acid **22**, which undergoes a spontaneous Lossen type rearrangement to yield rapalexin A (**4**). These chemical transformations, similar to the formation of isothiocyanates in plants, lend further support to the proposed rapalexin A biosynthetic pathway.[8] Likewise, transformation of 1-$MeSO_2$-glucorapassicin A (**9**) under acidic conditions yields 1-$MeSO_2$-rapalexin A (**11**). By contrast, in basic media (K_2CO_3/MeOH), a skeletal rearrangement of 1-*t*-Boc-glucorapassicin A (**10**) and 1-$MeSO_2$-glucorapassicin A (**9**) yields the unique product X (**14**), likely via the azirine intermediate **23** that undergoes a Neber type rearrangement upon nucleophilic attack by the HO-(C-2) of glucose. This transformation appears to generate the first preparation of a 1-deoxy-1-thioimidocarbonyl-β-D-glucopyranose heterocyclic ring system.[18] Recently, reactions of silyl-protected enol diazoacetates with nitrile oxides were reported to yield rearrangement products via dipolar cycloadditions followed by either Neber or Lossen rearrangements.[19]

In summary, the first biomimetic synthesis of rapalexin A (**4**) instigated a novel one-pot preparation that revealed a novel application of the Lossen rearrangement. Furthermore, the first bicyclic glucose-fused product (**14**) formed via Neber rearrangement was discovered. The scope of these transformations and other approaches to synthesize glucorapassicin A (**2**) are currently under investigation.

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