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Chemical Synthesis of natural and artificial glucosinolates. New Tools for analytics and biology

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In the field of animal breeding, glucosinolates (GSL) are rightly considered as antinutritional factors, the amount of which in rapeseed meal has to be kept below a threshold set by CEC regulations. With regard to the physiological effects they cause, GSL are generally considered as a whole structurally homogeneous family and referred to as "total glucosinolates".

Although the essential role played by the structure of the lateral chain of a GSL has long been known, the study, and hence the elaboration of molecularly pure individual GSL, remains relatively uncommon and is based most of the time on extraction and purification techniques applied to plant material.

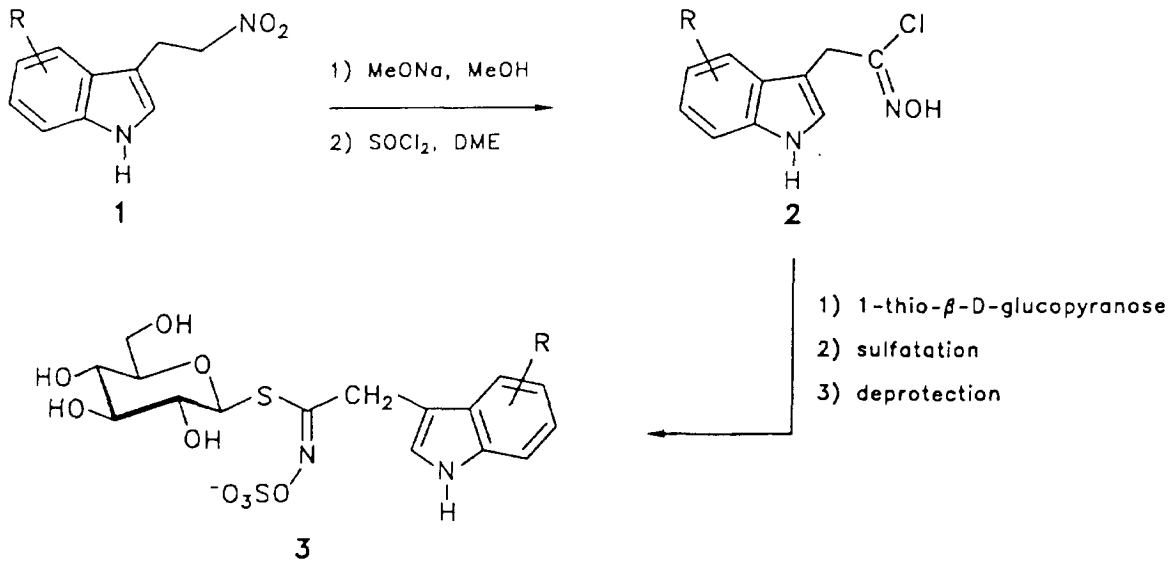
While the latter approach is well suited to the case of most "classical" GSL (sinigrin, progoitrin, gluconapin), which exhibit reasonable stability, in the case of other GSL such as the indole-derived GSL, which are rather oxygen-sensitive, chemical synthesis has proven particularly efficient in opening access to glucobrassicin and some of its derivatives.

Furthermore, chemical synthesis is the only possible way to produce artificial GSL with a view to their diverse analytical and biological applications. Consequently, GSL structures can be produced which are tailored to specific uses.

1. Synthesis of indole GSL

In view of the presumed carcinoprotective role of glucobrassicin .ans its metabolites[1], opening access through synthesis to indole GSL, the least well-known GSL family, is of considerable importance.

A general strategy for such synthesis has been developed in our laboratory[2], the key-step in which is the coupling of 1-thio-β-D-glucopyranose with an indole hydroximoyl chloride **2**, derived in turn from a primary nitro precursor **1**.



R= H, 4-OMe, 5-OMe, 5-Br

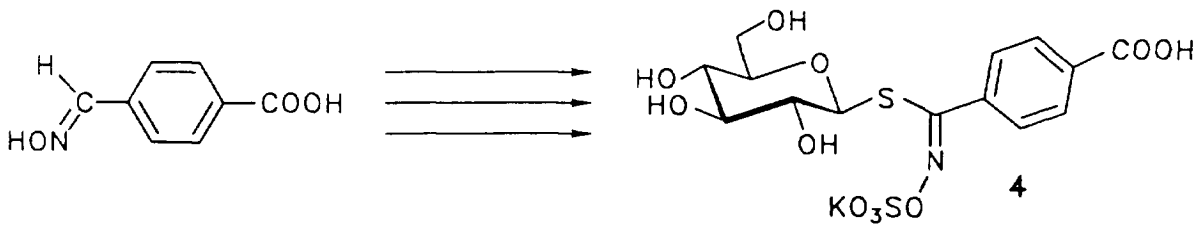
The intermediate glucopyranosyl thiohydroximate then undergoes sulfation with the pyridine-sulfur trioxide complex, and finally deprotection of the D-gluco unit yields the desired indole GSL **3**.

This method has enabled the production in sub-gram quantities of glucobrassicin and some of its methoxylated[3] or brominated[4] derivatives intended for diverse applications. Other substituted or modified glucobrassicins could be prepared in the same way from suitable indole precursors.

2. Synthesis of an immunogen for ELISA tests

The recent development of immunoenzymatic analysis methods in agrobiology[5] has led us to set up an ELISA (enzyme-linked immunosorbent assay) technique to determine the total GSL in rapeseed[6]. Anti-GSL polyclonal antibodies were obtained by immunizing rabbits with a BSA conjugate of the synthetic hapten **4**.

BSA (bovine serum albumin), a hydrosoluble protein, is a good carrier since its structure includes 59 lysine residues to which carboxylic functions can be grafted. Using the general method developed in our laboratory[7], 4-carboxybenzaldoxime is activated by chlorination with N-chlorosuccinimide then coupled with protected 1-thio-β-D-glucopyranose to give an intermediate thiohydroximate. Sulfation, followed by deprotection, yields 4-carboxyphenyl GSL **4**.

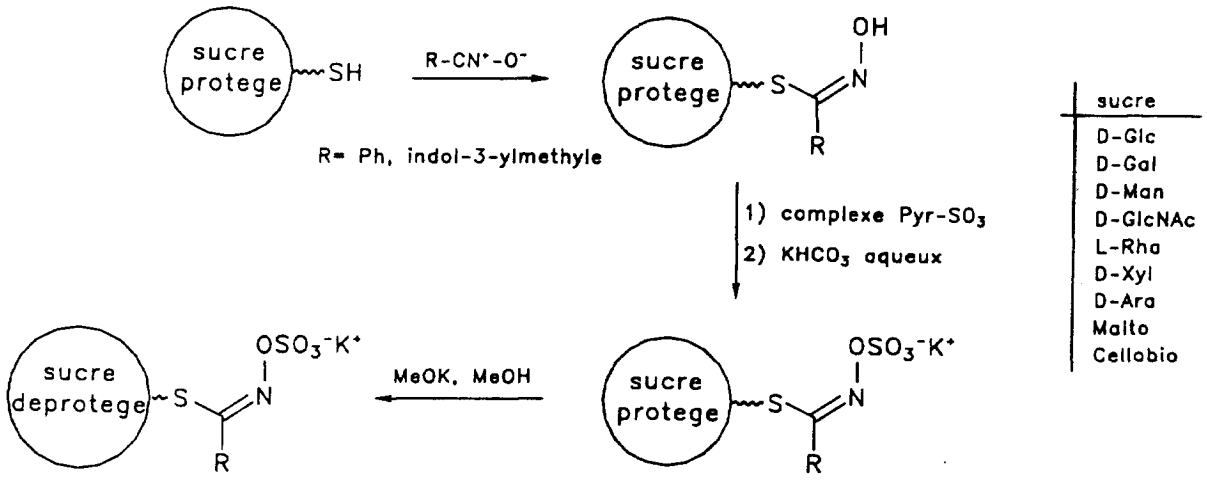


This artificial hapten, equipped with a COOH anchor, can then be attached to BSA using the classical EDCI method, to produce the desired immunogen.

3. Synthesis of glycosinolates

In order to carry out detailed investigations of myrosinase activity, a wide range of artificially modified GSL molecules is required, which, when confronted with the enzyme, can then enable an accurate assessment of the phenomena of recognition and inhibition.

Our work has specifically targetted the structural modifications which occur in the osidic moiety, while keeping the aglycon part constant. Thus, by replacing the D-gluco unit with sugars of different series, we have been able to construct a range of glycoviolins (R = phenyl)[8], as well as a similar range of glycobrassicins (R = indol-3-ylmethyl)[9].



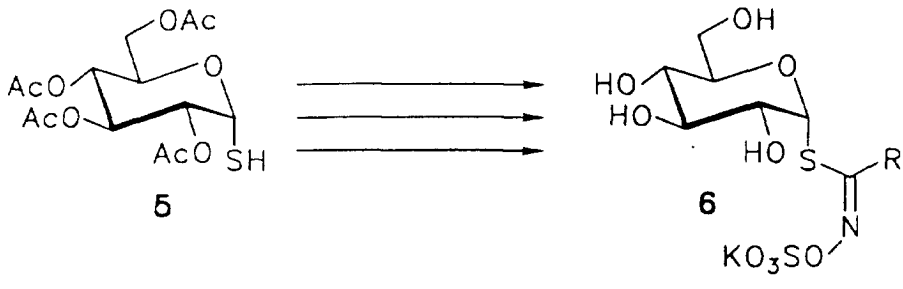
4. Synthesis of GSL with a modified D-gluco unit

With a view to studying further the active site of the enzyme, we subjected the D-glucopyrano unit to diversely "located" structural modifications, thereby presenting myrosinase with novel artificial substrates, in order to determine the relative importance of certain zones of the sugar portion of GSL in the recognition process.

4.1. Synthesis of alpha-GSL

Irrespective of any prospect of enzyme applications, the synthesis of alpha-anomerically configurated GSL is of undoubted theoretical significance, since GSL present in plants are exclusively beta-anomers.

Synthesis[10] followed the procedure described above, the sole difference being to replace the beta-thiol by its alpha anomer **5**, which was prepared according to Driguez et al.[11]



5 6

R= $PhCH_2$, $PhCH_2CH_2$, indol-3-ylmethyle

The key stage - addition of the thio-sugar unit to the intermediate nitrile oxides - is also stereospecific and produces only α-thiohydroximates which exhibit a strictly Z configuration, as shown by X-ray crystallography.[12]

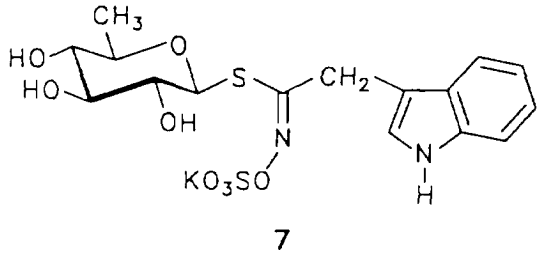
This sequence has thus enabled the synthesis of artificial GSL 6, a-anomers of glucotropaeolin, gluconasturtiin and glucobrassicin.

4.2. Synthesis of deoxy-GSL

The study of inhibitions attributable to the chemical modification of an oxygenated site on an osidic unit is a now classical approach : it was therefore only natural to attempt to apply it to the case of GSL.

Here again, opening access by synthesis to partly deoxygenated GSL presupposes obtaining access to the corresponding thiol precursors.

By way of example, two major syntheses have been achieved so far : namely, 6-deoxyglucobrassicin 7 and 2-deoxyglucobrassicin 8.[13]



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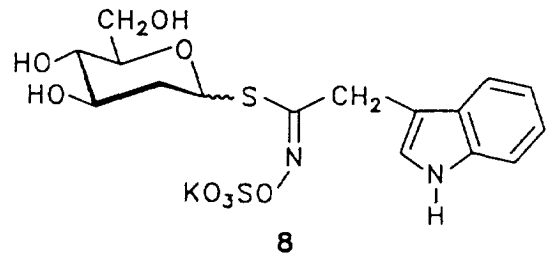
6-desoxyglucobrassicine



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2-desoxy-(α,β)-glucobrassicine

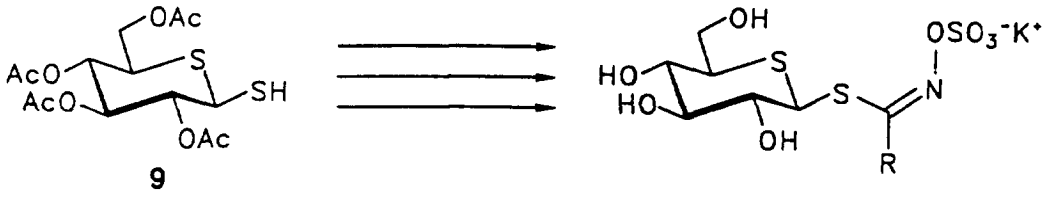
In the first case, the synthesis of the precursor 1-thio-b-D-quinovose calls on a modified version of the method described elsewhere in the literature.[14]

In the second case however, access had first to be opened to the previously unknown 2-deoxy-1-thio-D-glucopyranose, using the procedure developed by Thiem et al.[15]

The synthesis sequence studied exhibited at every stage considerable problems of stereoselection due mainly to the absence of an oxygenated site in the immediate vicinity of the anomeric carbon, with the result that the 2-deoxyglucobrassicin prepared was obtained in the form of a 1:1 mixture of alpha and beta anomers.

4.3. Synthesis of 5-thio-GSL

In this final case, the aim was to replace the endocyclic oxygen of the pyranose with a sulfur atom. The crucial step was, therefore, to synthesize the 5-thio-analogue of 1-thio-β-D-glucopyranose **9**. The sequence was achieved from 5-thio-D-glucose, which was prepared following the method of Driguez.[16]



9

10 R= $PhCH_2$

11 R= indol-3-ylmethyle

In this manner, two 5-thio-analogues of GSL - 5-thioglucotropaeoline **10** and 5-thioglucobrassicin **11** - were synthesized.[17] It should be noted that this is the first time that precursor **9** has been elaborated, since no representative of this family of 1,5-dithioglycopyranoses has ever been described.

The above results show clearly to what extent bioorganic synthetic chemistry can prove a valuable additional tool in the study of natural glucosinolates.

Above all, however, they make it outstandingly clear that not only is the synthetic approach absolutely essential in order to create new tools in several fields - particularly analytics and biology - but also that producing tailor-made structures may frequently have significant repercussions in fundamental chemistry.

References

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