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Carba-glucotropaeolin: the first non-hydrolyzable glucosinolate analogue, to inhibit myrosinase

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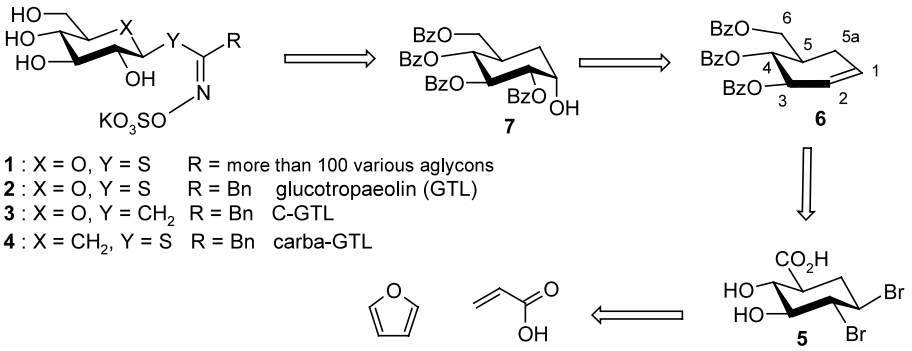
Abstract—A 5a-carba-analogue **4** of glucotropaeolin **2** was synthesized in racemic form and showed a good inhibiting power against myrosinase.

Glucosinolates **1** are naturally-occurring thiosugars mainly found in the botanical order *Brassicales*. The structural framework of glucosinolates invariably results from a combination of three segments: a D-glucopyranose unit, a *O*-sulfated anomeric thiohydroximate function and a broad library of aglycons, whose structure diversifies in the vegetal kingdom according to species.[1] Myrosinase (thioglucoside glucohydrolase EC 3.2.3.1) is the only enzyme able to hydrolyze those thiosaccharidic compounds. With a view to reaching a better understanding of the enzyme–substrate recognition mechanism, various analogues of glucosinolates have been synthesized. Substrate mimics have thus been prepared with structural modifications on the glycosidic moiety,[2] on the aglycon chain[3] or on the anionic site.[4] Most of these modifications proved useful in clearly demonstrating the specificity of myrosinase towards the D-glucopyrano moiety and the flexibility with regard to the aglycon moiety. So far, 2-fluoro-2-deoxy-glucotropaeolin[2f] was the only good inhibitor ever synthesized which allowed studies on the activity of myrosinase, notwithstanding a tendency to slow-rate hydrolysis.

For that reason, any X-ray analysis[5] of the myrosinase-substrate interaction was precluded.[2b] In order to improve our knowledge of the substrate conformation and binding inside the enzymatic pocket, a non-hydrolyzable substrate was therefore needed.

Glucotropaeolin (GTL) **2** was taken as a model to develop non-hydrolyzable substrates because of its current use as a EU official standard for the analysis of glucosinolates. We have published earlier the synthesis of a non-hydrolyzable *C*-glucoside analogue **3** (C-GTL), which unluckily showed no inhibition of myrosinase activity.[3b]



Scheme 1.

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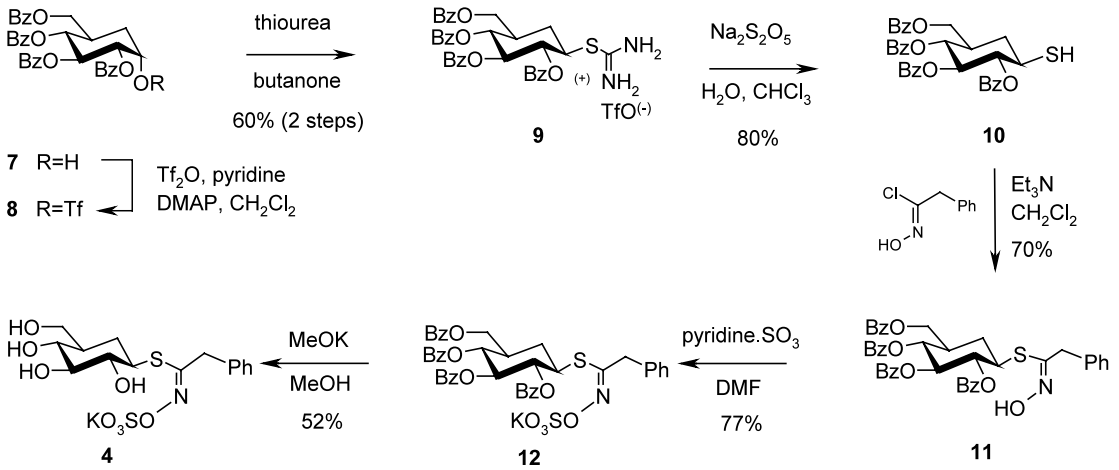

Scheme 2.

Another way to obtain a non-hydrolyzable substrate would consist in keeping in place the thiohydroximate function while replacing the endocyclic oxygen of the glucopyrano moiety by a methylene group, thus building up a 5a-carba-analogue **4** of GTL **2**. Our retrosynthetic strategy (Scheme 1) starts with the well known pathway developed by S. Ogawa:[6] Diels–Alder cycloaddition of acrylic acid on furan followed by cleavage of the ether bridge under acidic conditions [HBr, AcOH] gave the dibromo acid **5** which, after standard chemical transformations led to the protected 5a-carba-DL-glucal **6**. After dihydroxylation and selective protection of the hydroxyl in position two, the axial secondary alcohol **7** was obtained (Scheme 2).

Thiofunctionalization of the tetra-*O*-benzoylated 5a-carba-DL-glucose **7** was performed through conversion into the triflate **8** and nucleophilic displacement using thiourea as the sulfur donor. This method introduced sulfur with inversion of configuration as a pseudo-β-isothiouronium salt **9** which could be readily reduced with $Na_2S_2O_5$ to afford the thiol **10** with 80% yield. Under such conditions, formation of a symmetric disulfide was avoided. Thiol **10** was then reacted with the nitrile oxide generated in situ from benzhydroximoyl chloride to produce the thiohydroximate **11** in reasonable yield. *O*-Sulfatation of **11**, followed by deprotection gave DL-5a-carba-GTL **4** in 52% yield.[7]

Preliminary investigation of the inhibition properties of this carba-glucosinolate has been undertaken. An IC50 of 1 mM was determined for DL-carba-GTL thus showing a good inhibition activity, similar to that of 2-fluoro-2-deoxy-GTL.[2b] Compared to the non-inhibitory effect of the non-hydrolyzable analogue *C*-glycoside **2**,[3b] this result might be indicative of the importance of the sulfur atom in the interaction with myrosinase. Further work is under way to resolve the racemic mixture in order to be able to test each enantiomer separately with the enzyme.

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7. Spectral data for carba-GTL **4**.
^{1}H NMR (250 MHz, D_2O) δ (ppm): 1.14–1.40 (m, 3H, H-5, H-5a′, H-5a″), 3.12–3.31 (m, 4H, H-1, H-2, H-3, H-4), 3.44 (d, 1H, H-6a, $J_{6a-6b}=16.0$ Hz), 3.52 (d, 1H, H-6b), 4.05 (d, 1H, CH_2Ph, $J=16.8$ Hz), 4.12 (d, 1H, CH_2Ph), 7.31–7.48 (m, 5H, H arom.). **^{13}C NMR** (62.5 MHz, D_2O) δ (ppm): 32.7 (C-5a), 39.6 (CH_2Ph), 43.4 (C-5), 47.4 (C-1), 62.2 (C-6), 72.7, 75.2, 78.9 (C-2, C-3, C-4), 128.2, 129.1, 129.8, 135.9 (C-arom.), 165.4 (C=N). **ESMS** $m/z=406$ $(M-K)^-$.