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Synthetic Approaches to *C*-Glucosinolates

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Abstract—In these pages, short and efficient synthetic approaches to *C*-analogs of glucosinolates are described. Starting from D-glucose, *C*-glucotropaeolin (**6**) and *C*-glucocapparin (**11**) were synthesized in three steps. Preliminary enzymatic assays involving sulfatase and myrosinase have been performed.

Introduction

Glucosinolates constitute a large family of naturally occurring thiosugars found mainly in the botanical order *Brassicales*. They can be hydrolyzed by the enzyme myrosinase into D-glucose, hydrogen sulfate and various co-products depending on the structure of the aglycon.[1] Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) isolated from *Sinapis alba* ripe seeds is a glycoprotein containing various thiol groups and disulfide bridges together with ca. 18% carbohydrates, mainly hexoses. This enzyme consists of two identical subunits with a molecular weight of 71.7 kDa and it has a pI of 5.1.[2] Myrosinase is present in all glucosinolate-containing plants. Different types of glucosinolate analogs have been synthesized with the aim of explaining the mechanism of their enzymatic hydrolysis.[3] Glucotropaeolin (**1**) is the major glucosinolate isolated from plants of the genus *Nasturtium*. Using synthetic deoxy-glucotropaeolins[4,5] or 2-fluoro-2-deoxy-glucotropaeolin[6,7] and studying the myrosinase activity in comparison with native glucotropaeolin, the importance of the hydroxyl group at C-2 for glucosinolates binding was established and the molecular mechanism for its hydrolysis by myrosinase could thus be clarified. The design and synthesis of a non-hydrolyzable substrate such as a *C*-glycosidic analog would be of great interest in order to get additional information about the substrate conformation and binding into the active site of myrosinase by X-ray analysis.[8]

Keywords: glucosinolates; myrosinase inhibition; *C*-glycosides; Horner–Emmons reaction.

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Results and Discussion

Synthesis of *C*-glucosinolates

A brief retrosynthetic analysis called for a transient β-keto-*C*-glucoside which can be obtained following two different ways: either via an allyl-*C*-glucoside following a multistep route or via a Horner–Emmons reaction (Scheme 1).

We have previously reported[9] a synthesis of *C*-glucotropaeolin: following Kishi's procedure,[10,11] 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose was transformed into an allyl β-*C*-glycoside which led to the known epoxides **2** by *m*CPBA oxidation.[12] Regioselective epoxide opening of **2** by phenyllithium[13] afforded the diastereomeric mixture of alcohols **3**, which were further oxidized into ketone **4**. Hydrogenolytic deprotection of **4** afforded ketone **5** which was directly converted into *C*-glucotropaeolin (**6**) by condensation with hydroxylamine-*O*-sulfonic acid.[14] *C*-glucotropaeolin (**6**) was obtained as a 3:2 diastereomeric mixture of oxime sulfonates (ratio determined by ^{1}H NMR and confirmed by HPLC) (Scheme 2).

Although this synthetic pathway indeed allowed the preparation of *C*-glucotropaeolin (**6**), quite a number of steps were required, thus lowering the overall yield to 18% from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose. Alternatively, a more attractive approach to the key-compound **4** was to be developed as a one step Horner–Emmons type procedure.[15]

The synthesis of a dialkyl (2-oxo-3-phenyl)propanephosphonate was needed in order to obtain **4**. All the methods proposed in the literature for the synthesis of such β-keto phosphonates are rather tedious and inefficient.[16,17] Thus we tried to synthesize dimethyl (2-oxo-3-phenyl)propanephosphonate (**7**) following the method described by Mathey,[18] i.e. via the addition of the

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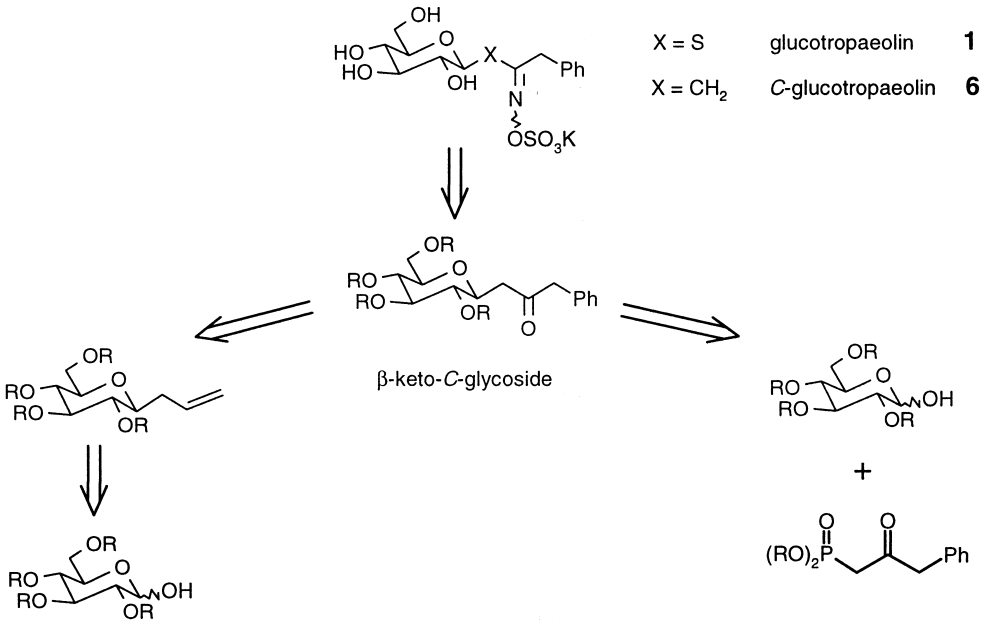


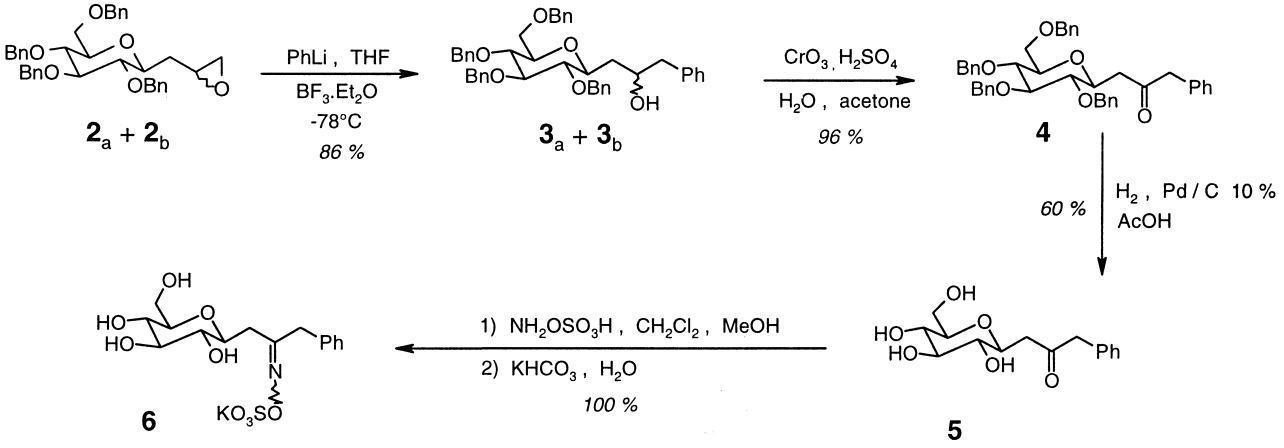
Scheme 1.

cuprate anion derived from dimethyl methanephosphonate on phenylacetyl chloride (Method A). The low reproducibility of this reaction led us to try another procedure[19] involving the condensation of the lithio derivative of dimethyl methanephosphonate on ethyl phenylacetate (Method B), but this latter reaction also proved disappointing. Finally, replacing the ester by the corresponding Weinreb amide[20,21] (Method C) allowed us to obtain **7** with good yields (Scheme 3).

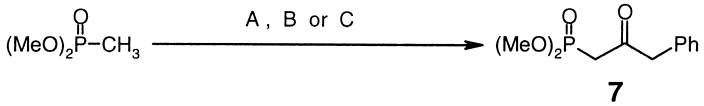
The first condensations of phosphonate **7** were tested on 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose. This approach led to poor results, whatever base (NaH, *n*BuLi, iPr_2NEt/$MgBr_2$, Et_3N/LiCl, MeONa, KOH) or solvent (THF, MeOH, H_2O/CH_2Cl_2, toluene, dioxane) used: yields inferior to 30% only were obtained. Application of heat to the reaction mixture promoted the expected side-reactions (C-2 epimerization,[22] β-elimination[23]). Moreover, a second step of epimerization of the α/β mixture into the β epimer was required consequently (Scheme 4).[24]

Compared to the first pathway (Scheme 1), the phosphonate condensation constitutes a much shorter route to the key compound **4** although the yield remains very low.

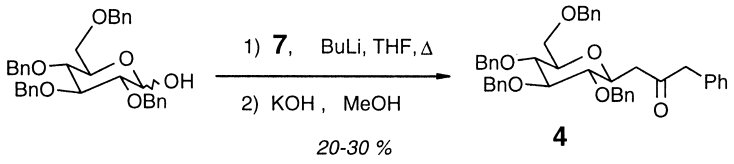
This poor reactivity could be attributed to the steric



Scheme 2.



Scheme 3. Methods. A: (i) *n*BuLi, THF; (ii) CuI; (iii) $PhCH_2COCl$ (0<yield<62%); B: (i) LDA 2 equiv., THF; (ii) $PhCH_2COOMe$ (31%); C: (i) *n*BuLi, THF; (ii) $PhCH_2CON(OMe)Me$; (iii) H_2O, HCl (79%).



Scheme 4.

hindrance of the benzyl group in 2-position.[25] In order to tide this problem over, we then tried to condense phosphonate **7** with 4,6-*O*-benzylidene-D-glucopyranose (**8**), which can be readily prepared in one step from D-glucose.[26] As a matter of fact, this reaction proved to be much more efficient and did not require any heating. In addition, the use of sodium methoxide in methanol allowed us to skip the epimerization step. The *C*-glycoside **9** was concomitantly deprotected and converted into the oxime sulfonate in a one-pot procedure using hydroxylamine-*O*-sulfonic acid. The diastereomeric ratio observed for *C*-glucotropaeolin (**6**) was similar to that previously obtained (3:2) (Scheme 5).

In order to check a possible general application of this method, we have made use of the same reaction protocol with **8** using commercially available diethyl 2-oxopropanephosphonate. By this way, we could obtain the *C*-glycosidic analog **11** of glucocapparin—a glucosinolate present in *Boscia senegalensis* and capers (*Capparidaceae*)—in comparable yields (Scheme 6).

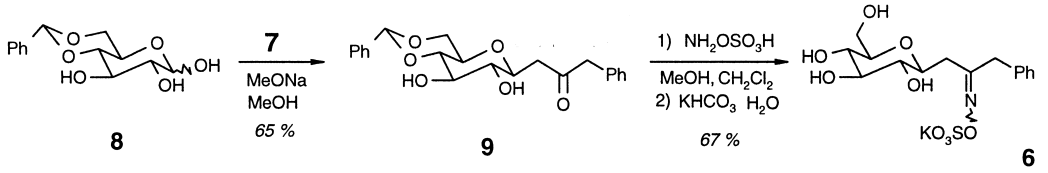
Analysis of *C*-glucosinolates

HPLC experiments on intact *C*-glucotropaeolin (**11**) have already been performed:[9] it was thus shown that an equilibrium existed between *E* and *Z* isomers under acidic conditions. The EU homologated analytical method for glucosinolates in rapeseed consists in an enzymatic desulfation on an ion exchange column, followed by the HPLC of the desulfated products on a C18 column (Scheme 7).[27,28] This preliminary desulfation allows to separate glucosinolates from the complex vegetable matrix.

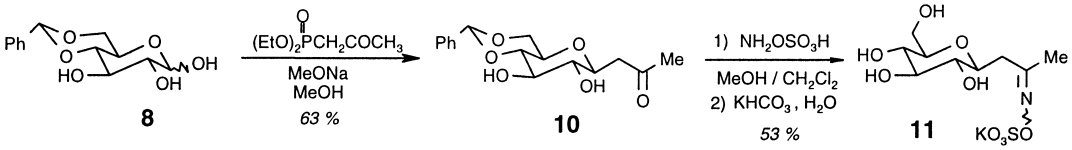
We have tried to apply this technique to *C*-glucotropaeolin (**6**). Unexpectedly, the chromatogram showed only one notable peak which might be either both stereoisomers, or only *Z* or *E*. In order to remove this ambiguity, we have performed the chemical synthesis of the non-sulfated diastereoisomers of *C*-glucotropaeolin (**14**) as standard compounds.

The direct conversion of ketone **5** into oxime **14** was ineffective. Therefore **5** was peracetylated and converted into its oxime derivative. A final transesterification step afforded the desired product **14** as a 3:2 diastereomeric mixture (ratio determined by ^{1}H NMR) (Scheme 8).

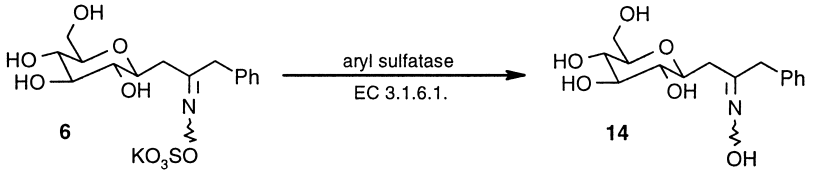
Co-injection of the diastereomeric mixture **14** together with the product resulting from enzymatic desulfation proved that only one isomer of the oxime was obtained in the enzymatic process. Moreover, a short study of the time-dependent evolution of **14** demonstrated that the two isomeric oximes were engaged in a slow equilibrium process, accompanied by hydrolysis to the corresponding



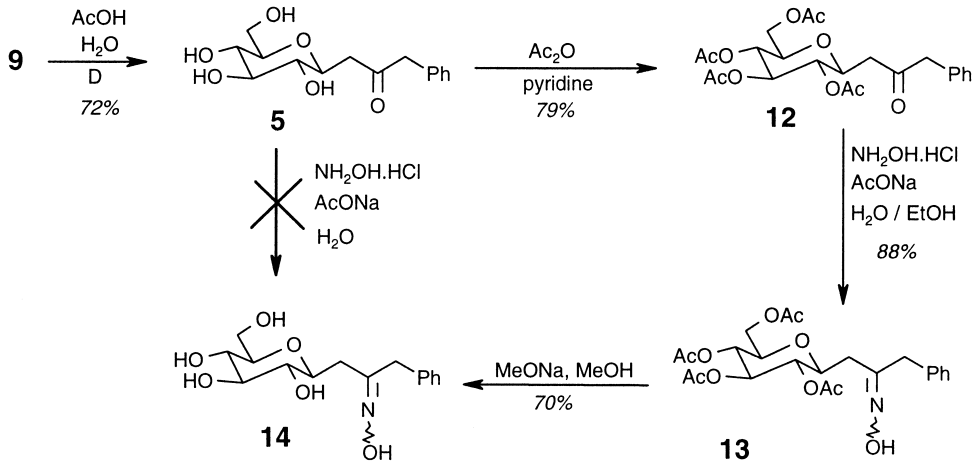
Scheme 5.



Scheme 6.



Scheme 7.



Scheme 8.

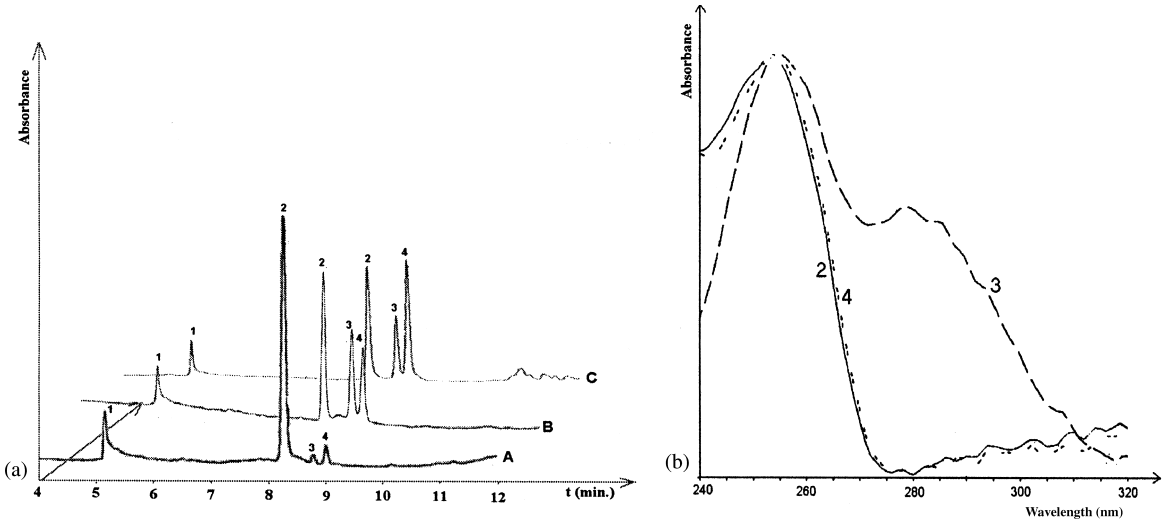


Figure 1. (a) Time-dependent evolution of the desulfated mixture. A: immediately after desulfation; B: 2 days after desulfation; C: 1 month after desulfation. Column: Spherisorb ODS-2 (250×4.6 mm); Mobile phase: CH_3CN in H_2O, 0–80 gradient in 20 min. (b) UV spectra of constituents 2, 3 and 4. Peak 1: unidentified impurity; Peak 2 and 4: desulfated isomers **14** of *C*-GTL; Peak 3: ketone **5**.

ketone (**5**) (Fig. 1a). UV absorption spectra of these three compounds are reported in Fig. 1b.

Myrosinase inhibition experiments

Preliminary investigation of the inhibition properties of the *C*-analogs have been undertaken. No activity against myrosinase has been detected so far: the diastereomeric mixture of *C*-glucosinolates is not degraded by the enzyme, and displays no inhibitory activity at concentrations up to 20 mM against sinigrin as substrate. These negative results seem to indicate that the *C*-glucosinolates do not bind to the enzyme. This is to our knowledge the first report showing that a glycosylhydrolase does not recognize the *C*-analog of its natural substrate.

Conclusion

We have described a simple access to *C*-glycosidic analogs of glucosinolates. This method, which can most likely be generalized, allows to synthesize *C*-glucosinolates in three steps, starting from D-glucose. Within this framework, we have developed an efficient route to dimethyl 2-oxo-3-phenylpropane phosphonate by use of a Weinreb amide. We have shown that the sulfatase which is currently used for the characterization of natural glucosinolates can recognise and desulfate our *C*-analogs, to afford only one of the two possible diastereoisomers. However, introductory inhibition experiments demonstrated that *C*-glucotropaeolin (**6**) was not recognized by myrosinase. Further work is in progress to find other analogs to explain the mechanism of myrosinase hydrolysis.

Experimental

General methods: Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX250 at 250 and 62.89 MHz, respectively, except for ^1H NMR spectra of compound **6** which were recorded on

a Bruker Avance DPX501 at 500 MHz. The chemical shifts (δ) are reported in ppm downfield from tetramethylsilane as the internal standard. Specific rotations were measured at 20°C using a Perkin–Elmer 141 polarimeter . IR spectra were measured using a Perkin–Elmer FT Paragon 1000 PC spectrophotometer. HRMS spectra were recorded on a VG analytical 70 SV. Evaporation, in vacuo, was conducted with a Büchi rotary evaporator. Analytical TLC was carried out on precoated silica gel 60F-254 plates (E. Merck) and spots were detected by UV light (254 nm). Flash column chromatography was performed on Kieselgel 60 (230–400 mesh) silica gel (E. Merck).

1-*C*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3-phenylpropan-2-ol (3). A solution of the diastereomeric mixture **2** (600 mg, 1.03 mmol)[12] in dry THF (50 mL) was cooled to −78°C and phenyllithium (1.14 mL, 1.8 M in hexane, 2 equiv.) then $BF_3{\cdot}Et_2O$ (0.27 mL, 2 equiv.) were added dropwise. The solution was stirred at −78°C for 35 min, quenched by the addition of methanol (30 mL) and was then allowed to warm to room temperature. The mixture was poured into a saturated aqueous solution of sodium bicarbonate and extracted with ethyl acetate (×3). The combined organic fractions were dried ($MgSO_4$), concentrated in vacuo and the residue purified by flash column chromatography (petroleum ether–AcOEt, 8:2) to afford a diastereomeric mixture of **3** (583 mg, 86%) as a white amorphous solid. Pure fractions of both isomers were isolated for characterization. Mp 108–110°C (**3a**), 68–70°C (**3b**); $[\alpha]_D$=+6 (**3a**), +13 (**3b**) (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$); diastereomer **3a**: δ 1.48 (ddd, 1H, H-1′b, $J_{1'b\text{-}1'a}$=14.5 Hz, $J_{1'b\text{-}1}$=9.7 Hz, $J_{1'b\text{-}2'}$=9.7 Hz), 2.06 (ddd, 1H, H-1′a, $J_{1'a\text{-}1}$=2.8 Hz, $J_{1'a\text{-}2'}$=2.8 Hz), 2.64 (dd, 1H, H-3′b, $J_{3'b\text{-}3'a}$=13.5 Hz, $J_{3'b\text{-}2'}$=7.3 Hz), 2.91 (dd, 1H, H-3′a. $J_{3'a\text{-}2'}$=6.2 Hz), 3.24 (dd, 1H, H-2, $J_{1\text{-}2}$=9.1 Hz, $J_{2\text{-}3}$=9.1 Hz), 3.38–3.84 (m, 6H, H-1, H-3, H-4, H-5, H-6a, H-6b), 4.01–4.17 (m, 1H, H-2′), 4.40–5.00 (m, 8H, C**H$_2$**Ph), 7.03–7.53 (m, 25H, H_{Ar}); diastereomer **3b**: δ 1.77 (ddd, 1H, H-1′b, $J_{1'b\text{-}1'a}$=14.5 Hz, $J_{1'b\text{-}1}$=7.2 Hz, $J_{1'b\text{-}2'}$=2.5 Hz), 1.91 (ddd, 1H, H1′a, $J_{1'a\text{-}1}$=3.5 Hz, $J_{1'a\text{-}2'}$=8.8 Hz), 2.72 (dd, 1H, H-3′b, $J_{3'b\text{-}3'a}$=13.5 Hz, $J_{3'b\text{-}2'}$=6.3 Hz), 2.83 (dd, 1H, H-3′a, $J_{3'a\text{-}2'}$=6.6 Hz), 3.34 (dd, 1H, H-2, $J_{1\text{-}2}$=8.8 Hz, $J_{2\text{-}3}$=8.8 Hz), 3.39–3.80 (m, 6H, H-1, H-3, H-4, H-5, H-6a, H-6b), 4.07–4.22 (m, 1H, H-2′), 4.32–4.98 (m, 8H, CH_2Ph), 7.05–7.45 (m, 25H, H_{Ar});^{13}C NMR ($CDCl_3$); diastereomer **3a**: δ 37.6 C-1′44.3 C-3′ 69.5 C-6; 73.7 C-2′ 74.0, 75.5, 76.0 C**H$_2$**Ph; 78.8, 79.1, 80.9, 87.2 C-1, C-3, C-4 and C-5; 82.6 C-2; 126.3, 126.7, 127.7, 128.1, 128.3, 128.4, 128.8, 128.9, 129.9, 138.0, 138.3, 138.9, 139.0 C_{Ar}; diastereomer **3b**: δ 37.2 C-1′ 44.3 C-3′69.3 C-6; 70.2 C-2′ 73.9, 75.4, 75.5, 76.0 CH_2Ph; 78.0, 78.7, 79.1, 87.6 C-1, C-3, C-4 and C-5; 81,4 C-2; 126.8, 128.1, 128.2, 128.3, 128.4, 128.8, 128.9, 129.9, 138.3, 138.4, 138.5, 138.9, 139.0 $C_{Ar;}$ HRMS: calcd for $C_{43}H_{46}O_6$ (658.3294), found (658.3303).

1-*C*-(2,3,4,6 -tetra-*O*-benzyl-β-D-glucopyranosyl)-3-phenylacetone (4). *Method A:* To a solution of the diastereomeric mixture **3** (500 mg, 0.76 mmol) in methanol (28 mL) cooled at 0°C Jones reagent (1.20 mL, 2.1 equiv.)[12] was added. The reaction was stirred at 0°C for 1 h and quenched by the addition of a 5% aqueous solution of sodium metabisulfite. The mixture was further diluted with a saturated aqueous solution of sodium bicarbonate and the aqueous layer extracted with ethyl acetate (×3). The combined organic extracts were dried ($MgSO_4$), concentrated in vacuo and the residue was recrystallized in ethyl acetate to give pure **4** (477 mg, 96%) as a white solid.

Method B: To a solution of phosphonate **7** (111 mg, 2.3 equiv.) in dry THF at 0°C was added *n*BuLi (0.58 mL, 1.6 M in hexane, 2 equiv.). The solution was stirred at this temperature for 30 min and 2,3,4,6 tetra-*O*-benzyl-D-glucopyranose (110 mg, 0.20 mmol) was added. The reaction mixture was refluxed for 5 h, quenched by water (10 mL) and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with brine, dried ($MgSO_4$), concentrated in vacuo and the residue was purified by flash column chromatography (petroleum ether–AcOEt) 9:1, followed by a recrystallization from ethyl acetate to afford ketone **4** (40 mg, 30%). Mp 74–76°C; $[\alpha]_D$=−3 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.60 (dd, 1H, H-1′b, $J_{1'b\text{-}1'a}$=15.3 Hz, $J_{1'b\text{-}1}$=8.3 Hz), 2.77 (dd, 1H, H-1′a, $J_{1'a\text{-}1}$=5.2 Hz), 3.33 (dd, 1H, H-2, $J_{1\text{-}2}$=9.4 Hz, $J_{2\text{-}3}$= 9.4 Hz), 3.44 (dt, 1H, H-5, $J_{4\text{-}5}$=9.6 Hz, $J_{5\text{-}6}$=3.0 Hz), 3.62–3.86 (m, 5H, H-1, H-3, H-4, H-6a, H-6b), 3.72 (s, 2H, H-3′), 4.47–5.00 (m, 8H, CH_2Ph), 7.10–7.48 (m, 25H, H_{Ar}); ^{13}C NMR ($CDCl_3$): δ 44.6 C-1′ 51.3 C-3′ 69.2 C-6, 73.9, 75.4, 75.9 CH_2Ph; 76.0 C-1; 78.8, 87.6 C-3 and C-4; 79.3 C-5; 81.5 C-2; 127.3, 128.1, 128.2, 128.3, 128.4, 128.8, 128.9, 129.0, 130.0, 134.4, 138.4, 138.5, 138.9 C_{Ar}; 206.4 CO; HRMS: calcd for $C_{43}H_{44}O_6$ (656.3138), found (656.3149).

1-*C*-(β-D-glucopyranosyl-3)-phenylacetone (5). *Method A:* A suspension of compound **4** (100 mg, 0.15 mmol) and 10% Pd/C (20 mg) in acetic acid (7 mL) was purged twice with hydrogen and stirred at room temperature for 22 h under 3 bar. The reaction mixture was then filtered through a pad of Celite, washed with acetic acid, concentrated in vacuo (coevaporation with toluene) and the residue was purified by flash column chromatography (AcOEt–MeOH–water, 90:8:2) to give ketone **5** (27 mg, 60%) as a colorless syrup.

Method B: Compound **9** (115 mg, 0.30 mmol) was dissolved in a 7:3 mixture of acetic acid and water (10 mL) and the resulting solution was heated at 60°C for 10 h. The yellowish oil obtained after evaporation of the solvent in vacuo was purified by flash column chromatography to afford ketone **5** (64 mg, 72%) as a colorless syrup. $[\alpha]_D$=−21 (*c* 1.0, MeOH);^{1}H NMR (CD_3OD): δ 2.64 (dd, 1H, H-1′b, $J_{1'b\text{-}1'a}$=15.8 Hz, $J_{1'b\text{-}1}$=8.9 Hz), 2.90 (dd, 1H, H-1′a, $J_{1b'a\text{-}1}$=2.8 Hz), 3.06 (dd, 1H, H-4, $J_{3\text{-}4}$=9.0 Hz, $J_{4\text{-}5}$=9.0 Hz), 3.17–3.39 (m, 2H, H-2 and H-3), 3.61–3.77 (m, 3H, H-1, H-6a and H-6b), 3.81 (dd, 1H, H-5, $J_{5\text{-}6}$=2.9 Hz), 3.88 (s, 2H, $H_{3'}$), 7.17–7.37 (m, 5H, H_{Ar}); ^{13}C NMR (CD_3OD): δ 48.1 C-1′; 49.3 C-3′; 60.9 C-6; 69.8 C-2; 73.2 C-4; 75.4 C-1; 77.7 C-3; 79.7 C-5; 125.9, 127.6, 128.9, 133.9 C_{Ar}; 207.5 CO; HRMS: calcd for $C_{15}H_{20}O_6$ (296.1260), found (296.1247).

1-*C*-β-D-glucopyranosyl-3-phenylacetone oxime *O*-sulfonate potassium salt (6). *Method A:* To a solution of compound **5** (100 mg, 0.34 mmol) in a 1:1 mixture of

dichloromethane and methanol (2 mL) at −10°C was added hydroxylamine-*O*-sulfonic acid (41 mg, 2.8 equiv.). The reaction was allowed to warm to room temperature, stirred for 15 min more and then hydrolyzed by a sodium bicarbonate solution (2.8 equiv.). The solvents were evaporated in vacuo and the residue was purified by flash column chromatography (AcOEt–MeOH–water, 80:15:5) then freeze-dried to give the *E*+*Z* diastereomeric mixture **6** (145 mg, quantitative) as a white amorphous solid.

Method B: Using the same procedure, compound **9** (200 mg, 0.52 mmol) was converted into the desired product **6** (150 mg, 67%). Mp 100–102°C (decomposition); ^{1}H NMR (CD_3OD): major isomer (M) **6a**: δ 2.39 (dd, H-1′;b, $J_{1'b\text{-}1'a}$=14.7 Hz, $J_{1'b\text{-}1}$=9.9 Hz), 2.81 (dd, H-1′a, $J_{1'a\text{-}1}$=2.9 Hz), 2.87–2.94 (m, H-5), 3.06–3.16 (m, H-2), 3.25–3.35 (m, H-3 and H-4), 3.39 (ddd, H-1, $J_{1\text{-}2}$=9.9 Hz), 3.54 (dd, H-6b, $J_{5\text{-}6b}$=4.8 Hz, $J_{6a\text{-}6b}$=12.9), 3.63 (dd, H-6a, $J_{5\text{-}6a}$=2.1 Hz), 3.77 (d, H-3′b, $J_{3'b\text{-}3'a}$=14.2 Hz), 3.93 (d, H-3′a), 7.25–7.40 (m, H_{Ar}); minor isomer (m) **6b**: δ 2.55 (dd, H-1′b, $J_{1'b\text{-}1'a}$=14.2 Hz, $J_{1'b\text{-}1}$=10.1 Hz), 2.74 (dd, H-1′a, $J_{1'a\text{-}1}$=2.9 Hz), 3.06–3.16 (m, H-2 and H-5), 3.25–3.35 (m, H-3 and H-4), 3.44 (ddd, H-1, $J_{1\text{-}2}$=10.1 Hz), 3.60 (dd, H-6b, $J_{6b\text{-}6a}$=12.3 Hz, $J_{5\text{-}6b}$=5.3 Hz), 3.68 (d, H-3′b, $J_{3'b\text{-}3'a}$=14.9 Hz), 3.72 (dd, H-6a, $J_{5\text{-}6a}$=2.1 Hz), 3.74 (d, H-3′a), 7.25–7.40 (m, H_{Ar}); ^{13}C NMR ($CDCl_3$): δ 29.9 C-1′$_m$; 33.5 C-1′$_M$; 35.3 C-3′$_M$; 38.9 C-3′;$_m$; 59.3, 59.6 C-6; 68.3, 68.5, 76.1 C-3 and C-4; 72.3, 72.8 C-2; 74.6, 75.1 C-1; 78.1, 78.3 C-5; 126.0, 126.2, 127.8, 127.9, 128.1, 132.6, 134.0, 134.5 C_{Ar}; 164.6, 165.5 C-2′ HRMS: calcd for $C_{15}H_{20}KNO_9S$ (429.0496), found (429.0488).

Dimethyl (2-oxo-3-phenyl)propane phosphonate (7). *Method A:* A solution of *n*BuLi (10.3 ml, 1.6 M in hexane, 1.1 equiv.) in dry THF (10 mL) was cooled to −78°C and dimethyl methanephosphonate (1.63 mL, 15 mmol) dissolved in dry THF (5 mL) was slowly added. The solution was stirred for 20 min at this temperature then freshly purified dry CuI (2.13 g, 1.13 equiv.) was added and the mixture was slowly warmed to −30°C. The dark suspension was stirred for 90 min then cooled to −40°C. Phenylacetyl chloride (2.12 mL, 1.05 equiv.) was then added and the reaction mixture was allowed to reach room temperature overnight. After dilution with water (15 mL) and CH_2Cl_2 (30 mL), the mixture was filtered through a pad of Celite (×2) and extracted with CH_2Cl_2 (×3). The combined organic extracts were dried ($MgSO_4$), concentrated in vacuo and the residue was purified by distillation under 1 Torr pressure (T_{eb}=120°C) to give phosphonate **7** (2.25 g, 62%) as a yellowish oil.

Method B: A solution of freshly distilled diisopropylamine (4.71 mL, 2.1 equiv.) in dry THF (40 mL) was cooled to −78°C and *n*BuLi (20 mL, 1.6 M in hexane, 2.0 equiv.) was added slowly. The reaction mixture was warmed up to 0°C, stirred at this temperature for 40 min then cooled to −78°C. Dimethyl methanephosphonate (1.71 mL, 16 mmol) was added and the resulting solution was stirred at 0°C for 30 min then cooled again to −78°C. Methyl phenylacetate (2.53 mL, 1.1 equiv.) dissolved in dry THF (5 mL) was added over a period of 30 min, then the solution was allowed to stir at room temperature for 18 h, quenched by the addition of a 1 M aqueous solution of HCl until neutral and extracted with ethyl acetate (×3). The combined organic extracts were washed with brine, dried ($MgSO_4$), concentrated in vacuo and the residue was purified by distillation under reduced pressure to afford phosphonate **7** (1.20 g, 31%) as a yellowish oil.

Method C: A solution of *n*BuLi (0.65 mL, 2.05 M in hexane, 0.9 equiv.) in dry THF (1.5 mL) was cooled to −78°C and dimethyl methanephosphonate (0.158 mL,1.46 mmol) in solution in dry THF (0.5 mL) was added. The deprotonation reaction was allowed to continue at 0°C for 20 min then the mixture was cooled again to −78°C. *N*-methoxy-*N*-methylphenylacetamide (240 mg, 1.34 mmol) was added and the solution was stirred at −78°C while monitoring by TLC. After 30 min, the mixture was hydrolyzed by the addition of 1 M aqueous HCl (5 mL) and extracted with CH_2Cl_2 (×3). The combined organic extracts were dried ($MgSO_4$), concentrated in vacuo and the residue was purified by flash column chromatography (petroleum ether–AcOEt, 3:7) to afford phosphonate **3** (255 mg, 79%) as a yellowish oil. IR (KBr) 1721 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 3.11 (d, 2H, CH_2PO, $^2J_{H-P}$=22.6 Hz), 3.78 (d, 6H, OCH_3, $^3J_{H-P}$= 11.3 Hz), 3.90 (s, 2H, CH_2Ph), 7.17–7.40 (m, 5H, H_{Ar}); ^{13}C NMR ($CDCl_3$): δ 40.5 (d, CH_2PO, $^1J_{C-P}$=123 Hz), 51.2 (d, CH_2Ph, $^3J_{C-P}$=1.8 Hz), 53.3 (d, MeO, $^2J_{C-P}$= 6.7 Hz), 126.8, 129.2, 130.0, 131.1 C_{Ar}, 199.9 (d, CO, $^2J_{C-P}$=6.1 Hz); HRMS: calcd for $C_{11}H_{15}O_4P$ (242.0708), found (242.0720).

1-*C*-(4,6-*O*-benzylidene-β-D-glucopyranosyl)-3-phenylacetone (9). To a solution of phosphonate **7** (600 mg, 2.2 equiv.) in methanol (3.7 mL) at room temperature was added sodium methoxide (2.26 mL, 1 M in methanol, 2 equiv.). After stirring for 1 h, 4,6-*O*-benzylidene-D-glucopyranose (**8**) (300 mg, 1.12 mmol)[26] was added. The resulting mixture was stirred 17 h more, hydrolyzed (10 mL) and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether–AcOEt, 3:7) then recrystallized from ethyl acetate to give ketone **9** (273 mg, 63%) as a white solid. Mp 182–184°C; $[\alpha]_D$=+6 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.66 (dd, 1H, H-1′b, $J_{1'b\text{-}1'a}$=16.2 Hz, $J_{1'b\text{-}=1}$=7.9 Hz), 2.88 (dd, 1H, H-1′a, $J_{1'a\text{-}1}$=3.8 Hz), 3.33 (dd, 1H, H-2, $J_{1\text{-}2}$=9.6 Hz, $J_{2\text{-}3}$=9.0 Hz), 3.37–3.50 (m, 1H, H-4), 3.54–3.79 (m, 3H, H-3, H-5 and H-6b), 3.74 (s, 2H, CH_2Ph), 3.84 (ddd, 1H, H-1), 4.25 (dd, 1H, H-6a, $J_{6a\text{-}5}$=4.3 Hz, $J_{6a\text{-}6b}$=10.4 Hz), 5.48 (s, 1H, CHPh), 7.15–7.55 (m, 10H, H_{Ar}); ^{13}C NMR ($CDCl_3$): δ 45.0 C-1′; 51.3 C-3′; 69.1 C-6; 70.7 C-2; 74.6 C-5; 75.6 C-3; 76.3 C-1; 81.4 C-5; 102.3 CHPh; 126.7, 127.5, 128.8, 129.1, 129.7, 130.0, 134.1 and 137.4 C_{Ar}; 206.5 CO; HRMS: calcd for $C_{22}H_{24}O_6$ (384.1573), found (384.1579).

1-*C*-(4,6-*O*-benzylidene-β-D-glucopyranosyl) acetone (10). To a solution of diethyl 2-oxopropane phosphonate (832 mg, 2.3 equiv.) in methanol (3.7 mL) at room temperature was added sodium methoxide (3.9 mL, 1 M in methanol, 2.1 equiv.). After stirring for 1 h, 4,6-*O*-benzylidene-D-glucopyranose (**8**) (500 mg, 1.86 mmol) was added. The resulting mixture was stirred for 21 h more, hydrolyzed (15 mL) and extracted with CH_2Cl_2 (×3). The combined

organic extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether–AcOEt, 3:7) to afford ketone **10** (361 mg, 63 %) as a white solid. Mp 142–144°C; $[\alpha]_D=-23$ (*c* 1.0, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H,CH_3), 2.64 (dd, 1H, H-1′b, $J_{1'b\text{-}1'a}$=16.2 Hz, $J_{1'b\text{-}1}$=7.9 Hz), 2.89 (dd, 1H, H-1′a, $J_{1'a\text{-}1}$=3.6 Hz), 3.36 (dd, 1H, H-2, $J_{1\text{-}2}$=9.6 Hz, $J_{2\text{-}3}$= 9.0 Hz), 3.42–3.53 (m, 1H, H-4), 3.59–3.78 (m, 3H, H-3, H-5 and H-6b), 3.86 (ddd, 1H, H-1), 4.29 (dd, 1H, H-5, $J_{4\text{-}5}$=10.3 Hz, $J_{5\text{-}6}$=4.0 Hz), 5.50 (s, 1H, CHPh), 7.33–7.53 (m, 10H, H_{Ar}); ^{13}C NMR ($CDCl_3$): δ 30.9 CH_3; 46.1 C-1′; 68.7 C-6; 70.4 C-5; 74.2 C-4; 75.2 C-2; 75.9 C-1; 81.0 C-3; 101.9 CHPh; 126.3, 128.4, 129.3, 137.0 C_{Ar}; 206.7 CO; HRMS: calcd for $C_{16}H_{20}O_6$ (308.1260), found (308.1247).

1-*C*-β-D-glucopyranosylacetone oxime *O*-sulfonate potassium salt (11). Starting from ketone **10** (104 mg, 0.337 mmol), the procedure used for compound **6** produced a *E*+*Z* diastereomeric mixture of oxime sulfonates **11** (63 mg, 53%) as a white amorphous solid. Mp 130–134°C (decomposition); ^{1}H NMR (CD_3OD): δ 1.89 (s, CH_{3m}), 1.93 (s, CH_{3M}), 2.33 (dd, H-1′b_m, $J_{1'b\text{-}1'a}$=14.5 Hz, $J_{1'b\text{-}1}$=9.8 Hz), 2.50 (dd, H-1′ b_M, $J_{1'b\text{-}1'a}$=14.6 Hz, $J_{1'b\text{-}1}$=9.8 Hz), 2.70 (dd, H-1′ a_m, $J_{1'a\text{-}1}$=2.7 Hz), 2.79 (dd, H-1′ a_M, $J_{1'a\text{-}1}$=2.5 Hz), 3.08 (dd, H-2_M, $J_{1\text{-}2}$=9.8 Hz, $J_{2\text{-}3}$=9.8 Hz), 3.11 (dd, H-2_m, $J_{1\text{-}2}$=9.8 Hz, $J_{2\text{-}3}$=9.8 Hz), 3.20–3.77 (m, H-1, H-3, H-4, H-5, H-6a and H-6b); ^{13}C NMR (CD_3OD): δ 14.9 C-1′$_M$; 20.1 C-1′$_m$; 32.8 C-3′$_m$; 37.9 C-3′$_M$; 61.1 C-6; 70.1, 77.6,1 C-3 and C-4; 73.4, 73.8 C-2; 76.3, 76.6 C-1; 79.9 C-5; 166.3, 166.6 C-2′ HRMS: calcd for $C_9H_{16}KNO_9S$ (353.0183), found (353.0192).

1-*C*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-phenylacetone (12). A solution of compound **5** (151 mg, 0.510 mmol) in pyridine (4 mL) and acetic anhydride (2 mL, 40 equiv.) was stirred for 15 h at room temperature. The solvent was then removed in vacuo (coevaporation with toluene) and the residue purified by flash column chromatography (petroleum ether–AcOEt, 7:3) to yield ketone **12** (187 mg, 79%) as a white solid. Mp 90–92°C; $[\alpha]_D=-15$ (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.94, 1.97, 2.00, 2.04 (4 s, 12H, CH_3CO), 2.47 (dd, 1H, H-1′b, $J_{1'b\text{-}1'a}$=16.4 Hz, $J_{1'b\text{-}1}$=3.2 Hz), 2.75 (dd, 1H, H-1′a, $J_{1'a\text{-}1}$=8.7 Hz), 3.63 (ddd, 1H, H-5, $J_{5\text{-}4}$=9.8 Hz, $J_{5\text{-}6b}$=4.9 Hz, $J_{5\text{-}6a}$=2.3 Hz), 3.70 (s, 2H, CH_2Ph), 3.98 (ddd, 1H, H-1, $J_{1\text{-}2}$=10.0 Hz), 4.04 (dd, 1H, H-6b, $J_{6b\text{-}6a}$=12.3 Hz), 4.20 (dd, 1H, H-6a), 4.84 (dd, 1H, H-4, $J_{4\text{-}3}$=9.4 Hz), 5.00 (dd, 1H, H-2, $J_{2\text{-}3}$=9.4 Hz), 5.16 (dd, 1H, H-3), 7.12–7.33 (m, 5H, H_{Ar}); ^{13}C NMR ($CDCl_3$): δ 22.85, 22.98 CH_3CO; 46.0 C-1′; 53.2 C-3′; 64.3 C-6; 70.7 C-2; 73.8 C-4; 76.2 C-1; 76.3 C-3; 78.0 C-5; 129.4; 131.0; 131.7, 135.6 C_{Ar}; 171.8; 172.1; 172.5; 172.9 $COCH_3$; 206.9 CO; HRMS: calcd for $C_{23}H_{28}O_{10}$ (464.1682), found (464.1675).

1-*C*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-phenylacetone oxime (13). Hydroxylamine hydrochloride (240 mg, 14 equiv.) and sodium acetate (480 mg, 23 equiv.) were dissolved in water (2 mL) and ketone **12** (116 mg, 0.25 mmol) was added. The suspension was diluted with ethanol (4 mL) until the mixture was homogeneous. After 45 min stirring at room temperature, a white precipitate was filtered then recrystallized in ethanol to give a diastereomeric mixture of oximes **13** (105 mg, 88%) as a white solid. Mp 70–72°C; ^{1}H NMR ($CDCl_3$): δ 1.92–2.32 (m, CH_3CO, H-1′a and H-1′b), 3.45–4.23 (m, H-1, H-5, H-6a and H-6b), 4.66 (2 dd, H-4, $J_{3\text{-}4}$=9.3 Hz, $J_{4\text{-}5}$=9.3 Hz), 4.87 (dd, H-2, $J_{1\text{-}2}$=9.3, $J_{2\text{-}3}$=9.3 Hz), 5.20 (dd, H-3), 7.13–7.39 (m, H_{Ar}), 10.69 and 10.79 (2 bs, NOH); HRMS: calcd for $C_{23}H_{29}NO_{10}$ (479.1791), found (479.1802).

1-*C*-β-D-glucopyranosyl-3-phenylacetone oxime (14). To a solution of compound **13** (180 mg, 0.37 mmol) dissolved in 2 mL methanol at room temperature was added sodium methoxide (370 μL, 1 M in methanol, 0.1 equiv.). The reaction mixture was stirred for 20 h then made neutral with Amberlite IR-120 (H^+) and the solvent was removed in vacuo. The residue was purified by flash column chromatography (AcOEt/MeOH/water 85/10/5) to afford a 4:3 diastereomeric mixture of oximes **14** (82 mg, 70%) as a white amorphous solid. Mp 70–72°C; ^{1}H RMN (CD_3OD): major isomer **14a**: δ 2.30 (dd, H-1′b, $J_{1'b\text{-}1'a}$=14.6 Hz, $J_{1'b\text{-}1}$=10.0 Hz), 2.62–2.80 (m, H-1′a), 2.91–3.01 (m, H-4), 3.05–3.20 (m, H-2), 3.27–3.40 (m, H-1, H-5, H-6a and H-6b), 3.51–3.68 (m, H-3), 3.69–3.81 (m, H-3′b), 3.89 (d, $J_{3'b\text{-}3'a}$=14.8 Hz, H-3′a), 7.23–7.48 (m, 10H, H_{Ar}); minor isomer **14b**: δ 2.62–2.80 (m, H-1′a and H-1′b), 3.05–3.20 (m, H-2 and H-4), 3.27–3.40 (m, H-3), 3.42–3.50 (m, H-1), 3.51–3.68 (m, H-5 and H-6b), 3.69–3.81 (m, H-3′b and H-6a),3.89 (d, H-3′b, $J_{3'b\text{-}3'a}$=14.8 Hz), 7.23–7.48 (m, 10H, H_{Ar}); ^{13}C RMN ($CDCl_3$): δ 29.4, 33.1 C-1′ 36.3, 39.5 C-3′ 60.3, 60.5 C-6; 69.2, 69.4 C-2; 73.3, 73.7 C-4; 75.8, 75.9 C-1; 77.0, 77.1 C-3; 78.9, 79.2 C-5; 125.8, 126.5, 126.8, 128.6, 128.7, 128.8, 139.9, 136.5 C_{Ar}; HRMS: calcd for $C_{15}H_{21}NO_6$ (311.1369), found (311.1372).

Myrosinase inhibition assays: A couple of hexokinase/glucose 6-phosphate dehydrogenase was used to assay the formation of glucose during the hydrolysis of glucosinolate (sinigrin) with myrosinase. The transformation was checked at 340 nm. Reference assays without *C*-glucosinolate was realized with solutions of sinigrin (0.3 mM), myrosinase (6.10^{-4} U/mL), MES buffer (pH 6.5), $MgCl_2$ (3 mM), ATP (0.55 mM), NADP (0.72 mM), hexokinase (0.56 U/mL), glucose 6-phosphate dehydrogenase (0.35 U/mL) at 25°C. For inhibtion assays, *C*-glucosinolate was added to attain 10 and 20 mM in final solutions. Results are reported in the text discussion.

Analysis of C-glucosinolates: The ISO 9167-1 standardized method used to analyze glucosinolates in rapeseed was slightly modified for *C*-glucosinolates and applied in the following way. After binding on a DEAE Sephadex A-25 exchanger column (15×8 mm), the *C*-glucosinolates were desulfated by a sulfatase (type H1, Sigma), eluted by water and analyzed by reverse phase liquid chromatography with a Spherisorb ODS 2, 5 μm, 250×4.6 mm column. The elution gradient was carried out from 0 to 80% of acetonitrile in water in 15 min and the *C*-desulfoglucosinolates were detected by UV absorption at 230 nm. The UV spectra (200–350 nm) were collected on a diode-array type spectrometer (Thermo-Quest, Focus) and treated by the Spectacle (Thermo-Quest) software.

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