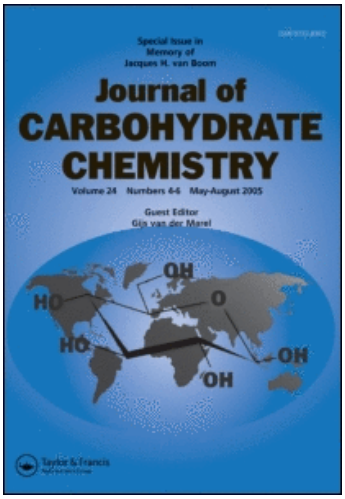

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SYNTHESIS OF CHLORAMPHENICOL AND MANDELONITRILE GALACTOSE-CONTAINING PRODRUGS

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ABSTRACT

The synthesis of O^1-β-D-galactopyranosylchloramphenicol and O^1-β-D-galactopyranosylmandelonitrile as prodrugs potentially substrates of β-galactosidase, are reported. Preparation of O^1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl) chloramphenicol from unprotected chloramphenicol was successful using β-D-galactopyranose pentaacetate and boron trifluoride diethyl etherate in acetonitrile. However, the β-galactosylated diastereoisomers of racemic mandelonitrile had to be made via O^1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)mandelamide in dichloromethane prior to dehydration to obtain the nitrile moiety. Indeed, galactosylation trials starting directly from mandelonitrile in acetonitrile led to the O^1-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)mandelonitrile diastereoisomers. From a methodological point of view, this work extends the use of the galactosylation method to new hydroxyl bearing compounds. It also points out that the solvent used (acetonitrile or dichloromethane) and the purity of boron trifluoride diethyl etherate can be crucial factors in the use of this method as an eventual

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alternative to heavy metal-based Lewis acids usually employed in glycosylation reactions.

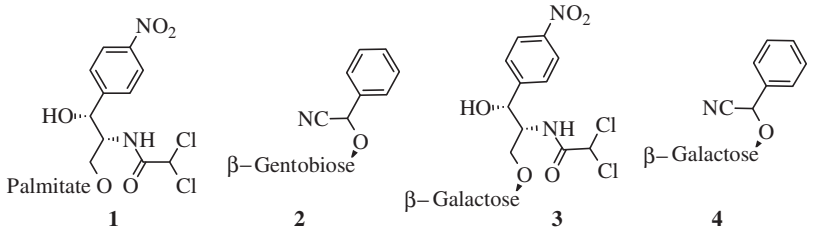
Key Words: Glycosylation; Galactosepentacetate; Prodrug; Mandelonitrile; Chloramphenicol

INTRODUCTION

The antibiotic chloramphenicol and the cyanide-generating mandelonitrile are both compounds displaying biological activity that can be (temporarily) blocked by a chemical transformation. Indeed, choramphenicol palmitate (**1**) has been developed[1] as an inert prodrug which is selectively hydrolysed by lipases thus releasing the antibacterial drug. Naturally occurring glycosylated derivatives of mandelonitrile, such as the gentobioside derivative amygdalin (**2**), are selectively hydrolysed by β-glucosidase, thus leading to the release of toxic cyanide.[2–4] Our current interest in drug targeting led us to elaborate prodrugs which would be specifically released by the action of β-galactosidase. Many successful examples of such prodrugs[5–9] have been reported for their potential application in selective chemotherapy, such as ADEPT[10,11] or GDEPT[12] (Antibody or Gene Directed Enzyme Prodrug Therapy). Thus, chloramphenicol or mandelonitrile β-galactosides **3** and **4** seemed good candidates for inactivation via galactosylation, since compounds **1** and **2** have proved to be prodrugs specifically activated by lipases or β-glucosidase, respectively (Figure 1).

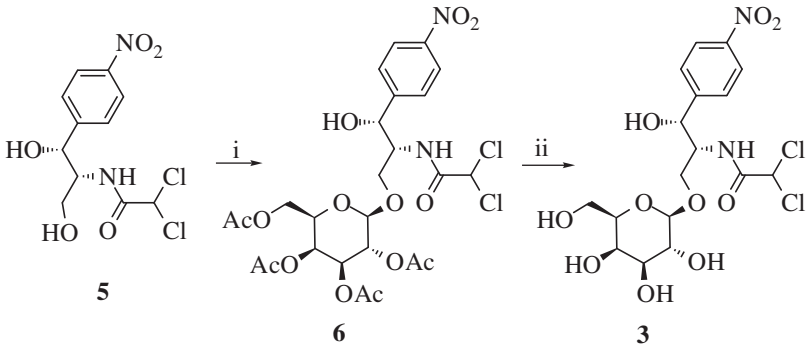
RESULTS AND DISCUSSION

The synthesis of prodrug **3** was achieved via the selective galactosylation of chloramphenicol **5**, using β-galactopyranose pentaacetate in the presence of boron trifluoride diethyl etherate in dry acetonitrile, to form the β-anomer **6** in 56% yield. The α-galactopyranose pentaacetate which is unreactive[13,14] toward these glycosylation conditions was also isolated. The regioselectivity of this galactosylation reaction was ascertained by NMR spectroscopy notably from the chloramphenicol methylene ^{1}H signals shift from 3.40 and 3.61 ppm to 3.54 and 4.01 ppm, respectively. Removal of



1 **2** **3** **4**

Figure 1.



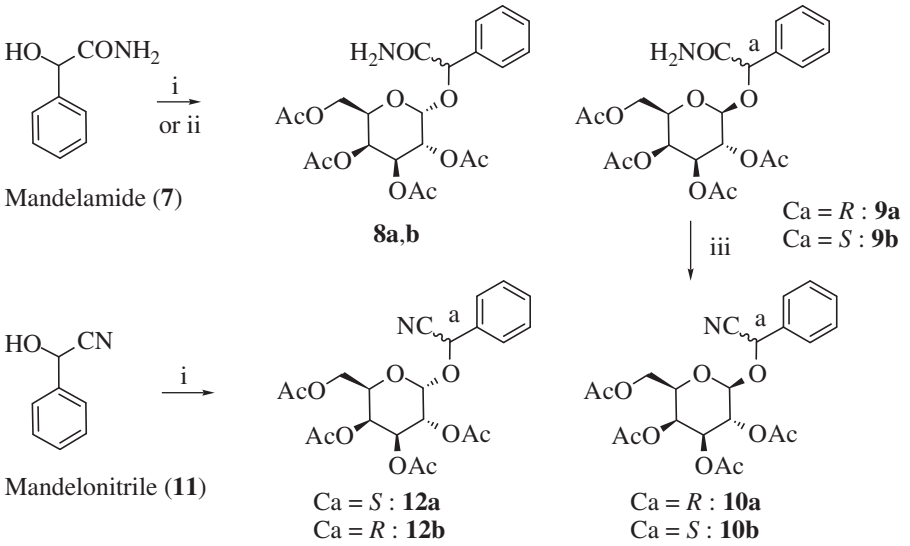
Scheme 1. i: β-D-Galactopyranose pentaacetate, $BF_3 \cdot OEt_2$, MeCN. ii: NH_3/MeOH.

the acetyl groups was then achieved using methanolic ammonia to give the galactoside **3** (Scheme 1).

Synthesis of compound **4** was far less straightforward. The reported preparation of mandelonitrile β-glucoside or β-glucuronide requires glycosylation of mandelamide **7** using tetraacetylglycopyranoside bromide and a mercuric salt.[15] In order to avoid the use of mercury, we focused on the galactosylation of mandelamide (**7**) using β-galactopyranose pentaacetate and boron trifluoride diethyl etherate complex.

Our first unsuccessful attempts led us to try the known β-galactosylation of benzyl alcohol. At first, using boron trifluoride diethyl etherate complex in dichloromethane, we were unable to reproduce the 72% yield previously reported.[16] As judged by TLC, the only observed result was an extensive decomposition. However, the β-galactosylated benzyl ether was prepared by simply changing the reaction solvent from dichloromethane to acetonitrile. Eventually, we found out that the purity of the boron trifluoride etherate was essential for this reaction. It was only by boiling boron trifluoride diethyl etherate and a small amount of diethyl ether over calcium hydride overnight, prior to its distillation, that we were able to obtain a Lewis acid pure enough for the reaction to proceed in dichloromethane (distilled over calcium hydride). On the other hand, if acetonitrile was used as solvent, the use of a boron trifluoride diethyl etherate of any reasonable purity or age led to an acceptable yield (55%) of β-galactosylated benzyl alcohol.

Dichloromethane is the solvent commonly used in this very simple galactosylation method which was first reported to give, from trichloroethyl alcohol, either the β-anomer[17] or, with a longer reaction time, the α-anomer primarily.[18] A later study,[13] with β-glucopyranose pentaacetate, illustrates some fascinating kinetic features of this method which seem highly dependent on the nature of the alcohol studied. Good β-glycosylation results have been obtained with different kinds of alcohols such as serine or threonine derivatives,[19–23] and even hydroxyproline.[24] Many other β-functionalised aliphatic alcohols can react[25–31] as well as benzyl,[16] allyl,[32–34] propargyl[35,36] or some other lipophilic alcohols.[37–39] The use of acetonitrile as solvent has been reported.[40] In some instances it was beneficial to the reaction outcome,[19] in another instance it was not beneficial.[22] Operating procedures usually favour the use of an excess or of an equivalent of boron trifluoride diethyl etherate. However, a remarkable 2-



Scheme 2. i: β-D-Galactopyranose pentaacetate, $BF_3{\cdot}OEt_2$, MeCN. ii: β-D-Galactopyranose pentaacetate, pure $BF_3{\cdot}OEt_2$, CH_2Cl_2. iii: $(CF_3CO)_2O$, pyridine, CH_2Cl_2.

deacetylation effect was reported[41] when an excess of allyl alcohol was present. In that case, the reaction led to the allyl 3,4,6-tri-*O*-acetyl-β-D-galactopyranoside in 62% yield.

In our cases, the reaction was carried out with an excess of the boron trifluoride diethyl etherate complex and we cannot over-emphasize the need for thorough purification of the Lewis acid, if dichloromethane is used. Acetonitrile and boron trifluoride may form a complex which allows the galactosylation reaction to proceed. However, at least one example of a chemical reaction dependent on the impurities content of the boron trifluoride diethyl etherate complex used has been reported.[42] Thus such impurities, which seem in our case to be detrimental to the galactosylation reaction in dichloromethane, are actually ''neutralized'' in acetonitrile.

In this context, we proceeded to try the galactosylation of mandelamide[43] **7**. In acetonitrile, using commercial boron trifluoride diethyl etherate, the reaction (i on Scheme 2) led to a complex mixture. From the ^{1}H NMR analysis of the partially purified material, the presence of the four possible diastereoisomers **8a**, **8b**, **9a** and **9b** was deduced, with predominance of the α-galactosides **8a** and **8b**. On the other hand, the galactosylation reaction (ii on Scheme 2) led mainly to the two β-diastereoisomers **9a** and **9b** in dichloromethane only if properly purified boron trifluoride diethyl etherate (as described above) was used. Diastereoisomer **9a** could be isolated via chromatography followed by recrystallization from 2-propanol in 33% yield whereas, despite repeated purification attempts, the oily diastereoisomer **9b** remained contaminated by small amounts of α-diastereoisomers **8a** and **8b**.

Each ^{1}H NMR spectra of the two β-anomers **9a** and **9b** displays a characteristic anomeric proton as doublets at 4.28 ppm or 4.62 ppm, with coupling constants of 7.9 Hz or 7.8 Hz, respectively. Traces of the α-anomers were also detectable in the reaction mixture mainly by their acetyl signals. The *S*-enriched mandelamide was prepared from

optically pure (*S*)-(+)-mandelic acid using a classical procedure[43] followed by recrystallization from toluene (ee[44] = 69%). Starting from this mandelamide, a large excess of compound **9b** was observed in the ^{1}H NMR spectra of the unchromatographed reaction mixture. This allowed us to assign the absolute configuration for all the β-configured derivatives, i.e. for **9a** and **9b** and, as described below, for compounds **10a** and **10b**.

A dehydration reaction[15] using pyridine and trifluoroacetic anhydride (iii on Scheme 2) was conducted on amide **9a** and on the fraction containing mostly **9b**. This enabled us to obtain the pure β-anomers **10a** and, from the fraction containing **9b**, the other β-diasteroisomer **10b** along with a small amount of the α-anomer **12b**.

Attempts to galactosylate mandelonitrile (**11**) with β-D-galactopyranose pentaacetate in acetonitrile (i on Scheme 2) led almost exclusively to the two α-diastereoisomers **12a** and **12b**. Diastereoisomer **12a** was isolated in 26% yield by recrystallization whereas the oily **12b** could only be partially purified by chromatography and still contained a small amount of **10a** and **10b**. Starting from (*R*)-(+)-enriched mandelonitrile (Aldrich, ee (in our hands) = 44%) the reaction led to a 2:3 mixture of compound **12a**:**12b** (as measured by their respective ^{1}H NMR signals integration ratio), thus allowing assignment of absolute configurations of compounds **12a** and **12b**.

Direct galactosylation of mandelonitrile in dichloromethane was unsuccesful, possibly because of nitrile chelation by boron trifluoride rendering its hydroxyl function unreactive. This seems reasonable as a similar lack of glycosylation was reported in trials using acetobromoglucose, mandelonitrile and mercuric salts.[15]

Deacetylation was conducted on β-anomer **10a**. Surprisingly, the use of methanolic ammonia led to partial amminolysis of the nitrile moiety along with racemization of the cyanohydrin asymmetric centre. The use of sodium methoxide in methanol was more efficient, but also led to the racemized target compound **4** as a hygroscopic syrup.

CONCLUSION

This work illustrates some additional results on the known use of boron trifluoride etherate as an alternative to heavy metal-based Lewis acids employed in glycosylation reactions. Preparation of the β-galactosyl-containing prodrug **3** via direct galactosylation of the primary hydroxyl function of chloramphenicol turned out to be very easy. On the other hand, our studies toward the preparation of compound **4** led to quite a lot more work. Indeed the galactosylation of mandelonitrile trials in dichloromethane and acetonitrile illustrates the subtlety of this reaction. In this work we observed the importance of employing an appropriate solvent (dichloromethane versus acetonitrile) and a pure catalyst (boron trifluoride), factors that one should be aware of in the course of trials carried out with this method. The potential use of prodrugs **3** and **4** in biology will be reported elsewhere.

EXPERIMENTAL

General methods. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 or Varian multi-400 or 500 spectrometers. Shifts are given in ppm with respect to

the TMS signal and coupling constants (J) are given in Hertz. Signals assignment was often confirmed by two dimensional NMR experiments (COSY, NOESY, HMQC, HMBCR). Low and high resolution mass spectra were obtained by Mrs Nicole Morin (ENS, 24 rue Lhomond F-75231 Paris) on a MS 700 Jeol and ammonia-based chemical ionization was used. Column chromatography was performed on Merck silica gel 60 (0.035–0.070 mm). Solvents were usually dried using activated 3 Å or 4 Å molecular sieves. Activation of the molecular sieves was done by using a plastic-free domestic microwave oven (irradiation in a quartz beaker of 100–200 g of new molecular sieves until partial melting, i.e. from 1 to 8 min by periods of 1 min alternated with cooling). **CAUTION**: due to residual traces of solvents, microwave irradiation of molecular sieves previously used can result in a serious explosion.

***O*^{1}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)chloramphenicol (6).** β-D-galactopyranose pentaacetate (2.5 g, 6.4 mmol) and chloramphenicol (12.4 g, 38.3 mmol) were dissolved in dry acetonitrile (300 mL). Boron trifluoride diethyl etherate (8 mL, 63.1 mmol) was added, and the solution was stirred under an inert atmosphere for 15 min. The solution was poured into water (500 mL), the organic materials were extracted with dichloromethane and the organic phase was dried over magnesium sulfate and concentrated to dryness. The residue was chromatographed over silica eluting with a mixture of heptane-ethyl acetate 4:5 yielding compound **6** as a hard foam (2.34 g, 56%). mp 85–89°C (dec.). ^{1}H NMR ($(CD_3)_2SO$, 400 MHz): 1.96, 2.04, 2.05, 2.16 (4s, 12 H, CH_3); 3.54 (dd, 1 H, J = 7.3 and 10, CH-1); 4.01 (dd, 1 H, J = 6 and 10, CH-1); 4.1 (m, 2 H, GalH-6); 4.11 (m, 1 H, CH-2); 4.25 (t, 1 H, J = 6.5, GalH-5); 4.82 (d, 1 H, J = 8.0, GalH-1); 4.92-5.05 (m, 2 H, GalH-2 and CH-3); 5.19 (dd, 1 H, J = 3.2 and 10.4, GalH-3); 5.31 (d, 1 H, J = 3.6, GalH-4); 6.21 (d, 1 H, J = 4.5 OH-3); 6.44 (s, 1 H, $CHCl_2$); 7.61 (d, 2 H, J = 5.8, CHAr-2); 8.22 (d, 2 H, J = 5.8, CHAr-3); 8.54 (d, 1 H, J = 8.8, NH). ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): 20.0 (CH_3); 54.6 (CH-2); 61.3 (GalC-6); 66.4 ($CHCl_2$); 67.4 (GalC-4); 67.7 (CH_2-1); 68.6 (GalC-2); 69.5 (CH-3); 70.0 (GalC-5); 70.2 (GalC-3); 100 (GalC-1); 123.0 (Ar-2); 127.4 (Ar-3); 146.6 (Ar-4); 150.4 (Ar-1); 163.4 (CONH); 169.2, 169.5, 169.9 ($COCH_3$). *m/z* (MH + NH_3) = 670. HRMS: Calcd for $C_{25}H_{34}N_3O_{14}{}^{35}Cl_2$: [$M^+ + NH_4$], 670.1417. Found: *m/z*, 670.1422.

***O*^{1}-β-D-Galactopyranosylchloramphenicol (3).** Compound **6** was treated with methanol saturated with ammonia (50 mL) in a sealed flask for 48 h at room temperature. The solution was concentrated to dryness to give compound **3** as a syrup. ^{1}H NMR ($(CD_3)_2SO$, 400 MHz): 3.37 (m, 1 H, CH-S3); 3.40 (m, 1 H, GalH-2); 3.44 (m, 1 H, GalH-5); 3.45 (m, 1 H, CH-1); 3.56 (m, 2 H, GalH-6); 3.70 (s (br), 1 H, GalH-4); 3.93 (t (br), 1 H, J = 9.0, CH-1); 4.13 (s(br), 1 H, CH-2); 4.22 (d, 1 H, J = 7.2, GalH-1); 5.22 (s, 1 H, CH-3); 6.51 (s, 1 H, $CHCl_2$); 6.21, 7.33 (2s (br), 2 H, 2 OH); 7.69 (d, 2 H, J = 8.7, CHAr-2); 8.19 (d, 2 H, J = 8.7, CHAr-3); 8.50 (m, 1 H, NH). ^{13}C NMR ($(CD_3)_2SO$, 100 MHz): 51.5 (CH-2); 57.5 (GalC-6); 63.6 ($CHCl_2$); 64.2 (CH_2-1); 65.3 (GalC-4); 66.1 (CH-3); 67.5 (GalC-5); 70.5 (GalC-3); 72.5 (GalC-2); 100.9 (GalC-1); 120.0 (Ar-2); 124 (Ar-3); 143.7 (Ar-4); 148.2 (Ar-1); 160.6 (CONH). *m/z* (MH + NH_3) = 502. HRMS: Calcd for $C_{17}H_{26}N_3O_{10}{}^{35}Cl_2$: [$M^+ + NH_4$], 502.0995. Found: *m/z*, 502.1007.

Preparation of compounds 9a and 9b. Mandelamide (6 g, 35.2 mmol) and β-D-galactopyranose pentaacetate (5.2 g, 13.3 mmol) were dispersed in dry dichloromethane (150 mL, distilled over calcium hydride) and the suspension was cooled to 0°C under an inert atmosphere. Very pure boron trifluoride diethyl etherate (11.8 mL, 93.1 mmol, refluxed with a small amount of ether over calcium hydride for 24 h prior to a distillation) was added and the solution was stirred overnight, allowing the temperature to rise back to 25°C. The solution was then poured into water and extracted with dichloromethane. The organic layer was cautiously washed with water, saturated sodium hydrogenocarbonate, water, then dried over magnesium sulfate and concentrated to dryness. The residue was chromatographed over silica gel eluting with a mixture of heptane-ethyl acetate with proportions gradually varying from 1:2 to 1:4. The fractions containing compounds **9a** and **9b** were recrystallized from 2-propanol yielding **9a** (2.17 g, 33%). The filtrate was concentrated to dryness and chromatographed over silica gel (eluting with heptane-ethyl acetate 1:4) to yield **9b** as a syrup (1.1 g, still containing small amounts of α-diastereoisomers).

***O*^{1}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(*R*)-mandelamide (9a).** mp 158°C. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.95, 2.00, 2.05, 2.15 (4s, 12 H, CH_3); 3.77 (t, 1 H, J = 7.0, CH-5); 4.14 (m, 2 H, GalH-6); 4.28 (d, J = 7.9, GalH-1); 4.91 (dd, 1 H, J = 3.4 and 10.5, GalH-3); 5.15 (s, 1 H, $HCCONH_2$); 5.25 (dd, 1 H, J = 7.8 and 10.5, GalH-2); 5.33 (m, 1 H, GalH-4); 5.53 and 6.81 (dependent on sample's concentration) (2s (br), 2 H, NH_2); 7.28–7.38 (m, 5 H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): 20.5, 20.6, 20.8 (CH_3); 61.2 (GalC-6); 66.9, 69.0, 70.2, 71.0 (GalC-3, GalC-2, GalC-4, GalC-5); 79.3 ($CHCONH_2$); 97.9 (GalC-1); 127.7 (CH-Ar); 128.8 (CH-Ar); 129.2 (CH-Ar); 135.1 (C-Ar); 170.0, 172.1 (CO and $CONH_2$).

Anal. Calcd for $C_{22}H_{27}NO_{11}$ (481.46): C, 54.88; H, 5.65; N, 2.91; O, 36.55. Found: C, 54.75; H, 5.63; N, 2.84; O, 36.56.

***O*^{1}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(*S*)-mandelamide (9b).** ^{1}H NMR ($CDCl_3$, 400 MHz): 1.95, 1.97, 2.06, 2.16 (4s, 12 H, CH_3); 3.81 (m, 1 H, GalH-5); 3.94 (m, 2 H, GalH-6); 4.62 (d, J = 7.8, GalH-1); 5.00 (dd, 1 H, J = 3.5 and 10.5, GalH-3); 5.08 (s, 1 H, $HCCONH_2$); 5.30 (m, 2 H, GalH-2 and GalH-4); 5.46 and 6.50 (dependent on sample's concentration), (2s (br), 2 H, NH_2) ; 7.30–7.42 (m, 5 H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): 20.6 (CH_3); 61.1 (GalC-6); 66.9, 69.3, 70.2, 70.9 (GalC-3, GalC-2, GalC-4, GalC-5); 81.1 ($CHCONH_2$); 100.8 (GalC-1); 127.1 (CH-Ar); 128.4 (CH-Ar); 128.8 (CH-Ar); 136.2 (C-Ar); 170.2, 172.6 (CO and $CONH_2$). *m/z* ($MH + NH_3$) = 499. HRMS: Calcd for $C_{22}H_{31}N_2O_{11}$: [$M^+ + NH_4$], 499.1927. Found: *m/z*, 499.1924.

Preparation of compound 10a and 10b. The crystalline amide **9a** (1.33 g, 8.8 mmol) was dissolved in dry dichloromethane (200 mL, distilled over calcium hydride). Pyridine (1.46 mL, 18.0 mmol, dried over 4 Å molecular sieves) and trifluoroacetic anhydride (1.27 mL, 9.0 mmol) were added. The solution was stirred under an inert atmosphere for 20 min. If TLC monitoring showed that the conversion was not completed, another portion of pyridine and trifluoroacetic anhydride were added. The organic solution was then poured into water, extracted with dichloromethane, washed

with water, dried over magnesium sulfate and concentrated to dryness. The residue was chromatographed over silica gel eluting with a 2:1 mixture of heptane and ethyl acetate, to provide compound **10a** as a hard foam (0.81 g, 63%). Using the same procedure, starting from 0.4 g of the fraction containing amide **9b** (still containing some α-diastereoisomers), 0.3 g of compound **10b** was obtained as an oil, along with small amount of **12b**.

***O*^{1}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(*R*)-mandelonitrile (10a).** mp 130°C. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.95, 1.97, 2.05, 2.10 (4s, 12 H, CH_3); 3.85 (t, 1 H, J = 6.7, GalH-5); 4.13 (m, 2 H, GalH-6); 4.47 (d, J = 8.0, GalH-1); 4.93 (dd, 1 H, J = 3.4 and 10.0, GalH-3); 5.26 (dd, 1 H, J = 8.0 and 10.0, GalH-2); 5.35 (d, 1 H, J = 3.4, GalH-4); 5.49 (s, 1 H, CHCN); 7.43 (m, 5 H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): 20.4 (CH_3); 61.0, 66.6, 68.2, 68.6, 70.5, 71.1 (GalC-6, GalC-4, GalC-3, GalC-2, GalC-5, CHCN); 99.0 (GalC-1); 116.6 (CN); 127.5 (CH-Ar); 129.1 (CH-Ar); 130.2 (CH-Ar); 132.1 (C-Ar); 169.8, 169.9, 170.0, 170.2 (CO). Anal. Calcd for $C_{22}H_{25}NO_{10}$ (463.44): C, 57.02; H, 5.44; N, 3.02; O, 34.52. Found: C, 57.05; H, 5.41; N, 2.89; O, 34.41.

***O*^{1}-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(*S*)-mandelonitrile (10b).** ^{1}H NMR ($CDCl_3$, 400 MHz): 1.97, 2.05, 2.07, 2.12 (4s, 12 H, CH_3); 4.01 (t, 1 H, J = 6.5, GalH-5); 4.17 (m, 2 H, GalH-6); 4.87 (d, J = 7.4, GalH-1); 5.07 (dd, 1 H, J = 3.4 and 10.2, GalH-3); 5.26 (dd, 1 H, J = 7.7 and 10.2, GalH-2); 5.42 (d, 1 H, J = 3.4, GalH-4); 5.66 (s, 1 H, CHCN); 7.45 (m, 5 H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): 20.45 (CH_3); 61.1, 66.7, 67.9, 68.1, 70.5, 71.3 (GalC-6, GalC-4, GalC-3, GalC-2, GalC-5, CHCN); 98.7 (GalC-1); 116.3 (CN); 127.0 (CH-Ar); 128.9 (CH-Ar); 129.9 (CH-Ar); 132.0 (C-Ar); 169.2, 169.8, 169.9, 170.2 (CO). *m/z* (MH + NH_3) = 481. HRMS: Calcd for $C_{22}H_{29}N_2O_{10}$: [$M^+ + NH_4$], 481.1822. Found: *m/z*, 481.1826.

Preparation of compound 12a and 12b. Mandelonitrile 85–90% (3.8 mL, 27.1 mmol) and β-D-galactopyranose pentaacetate (3 g, 7.6 mmol) were dispersed in dry acetonitrile (200 mL). Commercial boron trifluoride diethyl etherate (9.8 mL, 77.3 mmol) was added and the solution was stirred at 60°C for 30 min. The solution was then cautiously poured into a solution of sodium hydrogenocarbonate (25 g, 0.3 mol). The aqueous phase was extracted with dichloromethane, the organic layers were washed with water, dried over magnesium sulfate, then concentrated to dryness. The residue was chromatographed over silica gel, eluting with a mixture of heptane-ethyl acetate 3:2. The fraction containing compounds **12a** and **12b** (and to a much smaller extent isomer **10b**) were recrystallized from 2-propanol, yielding pure **12a** (0.92 g, 26%). The resulting filtrate was concentrated to dryness and chromatographed again over silica gel (eluting with heptane ethyl acetate 2/1) to give **12b** as a syrup (0.8 g, still containing a small proportion of **12a**).

***O*^{1}-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-(*S*)-mandelonitrile (12a).** mp 193°C. ^{1}H NMR ($CDCl_3$, 400 MHz): 1.95, 1.97, 2.06, 2.12 (4s, 12 H, CH_3); 4.08 (dd, 1 H, J = 6.5 and 11.8, GalH-6); 4.20 (dd, 1 H, J = 6.5 and 11.8, GalH-6); 4.43 (t, 1 H, J = 6.5, GalH-5); 5.09 (dd, 1 H, J = 3.8 and 11.0, GalH-2); 5.29 (d, 1 H, J = 3.8, GalH-1); 5.33 (s, 1 H, CHCN); 5.37 (dd, 1 H, J = 3.3 and 11, GalH-3);

5.51 (d, 1 H, J = 3.3, GalH-4); 7.43 (m, 5 H, Ar). ^{13}C NMR (DMSO*d6*, 100 MHz): 20.39, 20.4, 20.46, 20.57 (CH_3); 61.1 (GalC-6); 67.0, 67.57, 67.61, 67.7 (GalC-3, GalC-2, GalC-4, GalC-5); 68.5 (CHCN); 96.5 (GalC-1); 116.8 (CN); 127.0 (CH-Ar); 129.2 (CH-Ar); 130.3 (CH-Ar); 132.0 (C-Ar); 169.8, 169.9, 173.1, 173.3 (CO).

Anal. Calcd for $C_{22}H_{25}NO_{10}$ (463.44): C, 57.02; H, 5.44; N, 3.02. Found: C, 56.81; H, 5.39; N, 3.15.

***O*^{1}-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-(*R*)-mandelonitrile (12b).** ^{1}H NMR ($CDCl_3$, 400 MHz): 1.96, 2.04, 2.11, 2.13 (4s, 12 H, CH_3); 4.08 (m, 2 H, GalH-6); 4.13 (m, 1 H, GalH-5); 5.23 (m, 2 H, GalH-3 and GalH-2); 5.42 (m, 1 H, GalH-4); 5.45 (d, J = 3.0, GalH-1); 5.50 (s, 1 H, CHCN); 7.46 (m, 5 H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): 20.5 (CH_3); 61.4 (GalC-6); 66.9; 67.0; 67.5 (GalC-2, GalC-3, GalC-5 and GalC-4); 67.9 (CHCN); 95.1 (GalC-1); 116.4 (CN); 127.6 (CH-Ar); 129.2 (CH-Ar); 130.3 (CH-Ar); 132.0 (C-Ar); 169.8; 169.9; 170.1 (CO). *m/z* ($MH + NH_3$) = 481. HRMS: Calcd for $C_{22}H_{29}N_2O_{10}$: [$M^+ + NH_4$], 481.1822. Found: *m/z*, 481.1819.

***O*^{1}-β-D-Galactopyranosylmandelonitrile (4).** Compound **10a** (0.15 g, 0.3 mmol.) and sodium methanolate (0.04 g, 0.7 mmol) were stirred in dry methanol (30 mL, dried over 3 Å molecular sieves) under an inert atmosphere for 25 min. The solution was neutralized with Dowex 50, filtered, then concentrated to dryness to afford compound **4** as a hygroscopic mixture of the two β-diastereoisomers in a 60–40% proportion. ^{1}H NMR (CD_3OD, 500 MHz): 3.30 to 3.74 (m, 6 H, GalH-6, GalH-5, GalH-4, GalH-3, GalH-2); 4.26 (d, 2/5 H, J = 7.0, GalH-1 minor); 4.52 (d, 3/5 H, J = 7.0, GalH-1 major); 6.03 (s, 2/5 H, CHCN minor); 6.10 (s, 3/5 H, CHCN major); 7.52 (m, 3 H, CHAr major); 7.60 (m, 2 H, CHAr minor). ^{13}C NMR (CD_3OD, 125 MHz): Only small differences of signals were observed between the two diasteromers, thus hampering full assignment on their spectra: 60.2, 60.5, 66.7, 66.9, 67.8, 68, 70.3, 70.5, 73.0, 73.3, 75.8, 76 (GalC-2, GalC-3, GalC-4, GalC-5, GalC-6 and CHCN); 101 and 102 (GalC-1), 118 and 119 (CN), 127, 129, 129.6 (CHAr), 134 (CAr). *m/z* ($MH + NH_3$) = 313. HRMS: Calcd for $C_{14}H_{21}N_2O_6$: [$M^+ + NH_4$], 313.1399. Found: *m/z*, 313.1407.

REFERENCES

1. Edgerton, W.H.; Maddox, V.H.; Controulis, J. The structure of chloramphenicol palmitate. J. Am. Chem. Soc. **1955**, *77*, 27–29.
2. Haisman, D.R.; Knight, D.J. The enzyme hydrolysis of amygdalin. Biochem. J. **1967**, *103*, 528–534.
3. Fenselau, C.; Pallante, S.; Batzinger, R.P.; Benson, W.R.; Barron, W.R.; Sheinin, E.B.; Maienthal, M. Mandelonitrile β-glucuronide: Synthesis and characterisation. Science **1977**, *198*, 625–627.
4. Syrigos, K.N.; Rowlinson-Busza, G.; Epenetos, A.A. In vitro cytotoxicity following specific activation of amygdalin by β-glucosidase conjugated to a bladder cancer-associated monoclonal antibody. Int. J. Cancer **1998**, *78*, 712–719.
5. Abraham, R.; Aman, N.; von Borstel, R.; Darsley, M.; Kamireddy, B.; Kenten, J.; Morris, G.; Titmas, R. Conjugates of COL-1 monoclonal antibody and β-D-galac-

tosidase can specifically kill tumor cells by generation of 5-fluorouridine from the prodrug β-D-galactosyl-5-fluorouridine. Cell Biophys. **1994**, *24/25*, 127–133.
6. Leenders, R.G.G.; Damen, E.W.P.; Bijsterveld, E.J.A.; Scheeren, H.W.; Houba, P.H.J.; van der Meulen-Muileman, I.H.; Boven, E.; Haisma, H.J. Novel anthracycline-spacer-β-glucuronide, -β-glucoside, and -β-galactoside prodrugs for application in selective chemotherapy. Bioorg. Med. Chem. **1999**, *7* (8), 1597–1610.
7. Bakina, E.; Farquhar, D. Intensely cytotoxic anthracycline prodrugs: Galactosides. Anti-Cancer Drug Des. **1999**, *14*, 507–515.
8. Brüsselbach, S.; Korn, T.; Wölkel, T.; Müller, R.; Kontermann, R.E. Enzyme and recruitment and tumor cell killing in vitro by secreted bispecific single-chain diabody. Tumor Targeting **1999**, *4*, 115–123.
9. Ghosh, A.K.; Khan, S.; Marini, F.; Nelson, J.A.; Farquhar, D. A daunorubicin β-galactoside prodrug for use in conjunction with gene-directed enzyme prodrug therapy. Tetrahedron Lett. **2000**, *41* (25), 4871–4874.
10. Bagshawe, K.D. Towards generating cytotoxic agents at cancer sites. Br. J. Cancer **1989**, *60*, 275–281.
11. Syrigos, K.N.; Epenetos, A.A. Antibody directed enzyme prodrug therapy (ADEPT): A review of the experimental and clinical considerations. Anticancer Res. **1999**, *19*, 605–613.
12. Melton, R.; Connors, T.; Knox, R.J. The use of prodrugs in targeted anticancer therapies. S.T.P. Pharma Sci. **1999**, *9*, 13–33.
13. Ellervik, U.; Jansson, K.; Magnusson, G.J. Gas chromatographic investigation of the boron trifluoride etherate-induced formation and anomerization of glucopyranosides. J. Carbohydr. Chem. **1998**, *17* (4–5), 777–784.
14. Veerneman, G.H. Chemical Synthesis of *O*-Glycosides. In *Carbohydrate Chemistry*; Boons, G.-J., Ed.; Chapman and Hall: New York, 1998; 98–174.
15. Junghans, B. Darstellung des Cyanogen Glycosides Mandelglucuronid [2-(β-D-glucopyranuronosyloxy)-2-phenylacetonitrile]. Pharmazie **1982**, *37*, 172–175.
16. Kobayashi, Y.; Shiozaki, M.; Ando, O. Syntheses of trehazolin derivatives and evaluation as glycosidase inhibitors. J. Org. Chem. **1995**, *60* (8), 2570–2580.
17. Magnusson, G.; Noori, G.; Dahmén, J.; Frejd, T.; Lave, T. BF_3-etherate induced formation of 2,2,2-trichloroethyl glycopyranoside. Selective visualization of carbohydrate derivatives on TLC plates. Acta Chem. Scand., Ser. B **1981**, *35*, 213–216.
18. Risbood, P.A.; Reed, L.A., III; Goodman, L. The preparation and study of some novel glycosides of D-galactose. Carbohydr. Res. **1981**, *88* (2), 245–251.
19. Elofsson, M.; Walse, B.; Kihlberg, J. Building blocks for glycopeptide synthesis: Glycosylation of 3-mercaptopropionic acid and Fmoc amino acids with unprotected carboxyl groups. Tetrahedron Lett. **1991**, *32* (51), 7613–7616.
20. de la Torre, B.G.; Torres, J.L.; Bardají, E.; Clapés, P.; Xaus, N.; Jorba, X.; Clavet, S.; Albericio, F.; Valencia, G. Improved method for the synthesis of *O*-glycosylated Fmoc amino acids to be used in solid-phase glycopeptide synthesis. J. Chem. Soc., Chem. Commun. **1990**, 965–967.
21. Kihlberg, J.; Åhman, J.; Walse, B.; Drakenberg, T.; Nilsson, A.; Södeberg-Ahlm, C.; Bengtsson, B.; Olsson, H. Glycosylated peptide hormones: Pharmacological properties and conformational studies of analogues of [1-desamino, 8-D-arginin]-vasopressin. J. Med. Chem. **1995**, *38* (1), 161–169.
22. Salvador, L.A.; Elofsson, M.; Kihlberg, J. Preparation of building blocks for

glycopeptide synthesis by glycosylation of Fmoc amino acids having unprotected carboxyl groups. Tetrahedron **1995**, *51* (19), 5643–5656.

23. Sjölin, P.; Kihlberg, J. Deacetylation of *N*-α-methylated glycopeptides reveals that Aza-enolates provide protection against β-elimination of carbohydrates *O*-linked to serine. Tetrahedron Lett. **2000**, *41* (22), 4435–4439.
24. Arsequell, G.; Sàrries, N.; Valencia, G. Synthesis of glycosylated hydroxyproline building blocks. Tetrahedron Lett. **1995**, *36* (40), 7323–7326.
25. Dahmén, J.; Frejd, T.; Grönberg, G.; Lave, T.; Magnusson, G.; Noori, G. 2-bromoethyl glycosides: Synthesis and characterisation. Carbohydr. Res. **1983**, *116* (2), 303–307.
26. Tang, P.W.; Williams, J.M. The deamination of 1-aminohexan-2-ol and 1-(aminomethyl)pentyl β-D-galactopyranoside; a model study of the selective cleavage of the hydroxylysine-bound glycosyl residues of collagen. J. Chem. Soc., Perkin Trans. 1 **1984**, 1199–1203.
27. Sasaki, A.; Murahashi, N.; Yamada, H.; Morikaw, A. Syntheses of novel galactosyl ligands for liposomes and the influence of the spacer on accumulation in the rat liver. Biol. Pharm. Bull. **1995**, *18* (5), 740–746.
28. Chernyak, A.Y.; Sharma, G.V.M.; Kononov, L.O.; Khrisna, P.R.; Levinsky, A.B.; Kochetkov, N.K.; Roa, A.V.R. 2-azidoethyl glycosides: Glycosides potentially useful for the preparation of neoglycoconjugates. Carbohydr. Res. **1992**, *223*, 303–309.
29. Davis, B.G.; Lloyd, R.C.; Jones, J.B. Controlled site-selective glycosylation of proteins by a combinatorial site-directed mutagenesis and chemical modification approach. J. Org. Chem. **1998**, *63* (26), 9614–9615.
30. Davis, B.G.; Maughan, M.A.T.; Green, M.P.; Ullman, A.; Jones, J.B. Glycomethanethiosulfonates: Powerful reagents for protein glycosylation. Tetrahedron: Asymmetry **2000**, *11* (1), 245–262.
31. Suhr, R.; Scheel, O.; Thiem, J. Synthesis of glycosyl glycerols and related glycolipids. J. Carbohydr. Chem. **1998**, *17* (6), 937–968.
32. Lyubeshkin, A.B.; Anikin, M.V.; Gurine, A.V.; Sebyakin, Y.S. An alternative synthesis of 1,2-trans-glycosyl diglycerides. Russ. J. Org. Chem. **1994**, *30* (4), 604–609.
33. Mereyela, H.B.; Lingannagaru, S.R. A study of Pd(II)Cl2/CuCl catalysed wacker reaction for the deprotection of prop-2-enyl and prop-1-enyl ethers. Tetrahedron **1997**, *53* (51), 17501–17512.
34. Mandai, T.; Okumoto, H.; Oshitari, T.; Nakanishi, K.; Mikuni, K.; Hara, K.; Hara, K. A practical synthetic method for α- and β-glycosyloxyacetic acids. Heterocycles **2000**, *52* (1), 129–132.
35. Mereyala, H.B.; Gurrala, S.R. A highly diastereoselective, practical synthesis of allyl, propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-gluco-β-D-galactopyranosides and allyl, propargyl heptaacetyl-β-D-lactosides. Carbohydr. Res. **1998**, *307* (3–4), 351–354.
36. Mereyela, H.B.; Gurrala, S.R. Design, development and utility of glycosyl donors bearing and acetoxymethoxy leaving group. Chem. Lett. **1998**, 863–864.
37. Ansari, A.A.; Frejd, T.; Magnusson, G. 3-bromo-2-bromomethylpropyl glycosides in the preparation of double-chain bis-sulfide neo-glycolipids. Carbohydr. Res. **1987**, *161* (2), 225–233.
38. Susaki, H.; Suzuki, K.; Ikeda, M.; Yamada, H.; Watanabe, H.K. Synthesis of

artificial glycoconjugates of arginine-vasopressin and their antidiuretic activities. Chem. Pharm. Bull. **1994**, *42* (10), 2090–2096.

39. Ye, X.-S.; Wong, C.-H. Anomeric reactivity-based one-pot oligosaccharides synthesis: A rapid route to oligosaccharide libraries. J. Org. Chem. **2000**, *65* (8), 2410–2431.
40. Sivanandaiah, K.M.; Babu, V.V.S.; Shankaramma, S.C. Solid phase synthesis of *O*-glycoopioid peptides related to dermorphin. Indian J. Chem. **1998**, *37B*, 760–767.
41. Mei-Zheng, L.; Hong-Ni, F.; Zhong-Wu, G.; Yong-Zheng, H. One-step glycosylation and selective deprotection of peracetylated monosaccharides for facile syntheses of allyl glycosides with a free C-2 hydroxyl group. Carbohydr. Res. **1996**, *290* (2), 233–237.
42. Davies, J.A.; Petersohn, C.; Kukushkin, V.Y. Unprecedented formation of isoxazole in the reaction between nitromethane and BF3·OEt2 in the presence of ethylene. J. Chem. Soc., Perkin Trans. 1 **1998**, 3139–3140.
43. Audreith, L.F.; Sveda, M. Mandelamide. In *Organic Syntheses*; Horning, E.C., Ed.; John Wiley & Sons: New York, 1955; Vol. III, 536–538.
44. Myers, A.I.; Slade, J. Asymmetric addititon of organometallics to chiral ketooxazolines. Preparation of enantiomerically enriched α-hydroxy acids. J. Org. Chem. **1980**, *45* (14), 2785–2791.

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