A Novel General Route for the Synthesis of C-Glycosyl Tyrosine Analogues

Elisabetta Brenna,* Claudio Fuganti, Piero Grasselli, Stefano Serra, and Sabrina Zambotti[a]

Abstract: A general method for the synthesis of C-glycosyl amino acids is described here. The stereoisomerically pure tyrosine analogues α-L-**4** and β-L-**6** are prepared in reasonable overall yields from allyl derivatives **10** and **11**. The key step is a benzannulation procedure which is employed in the creation of the aromatic ring that bears the amino acid function.

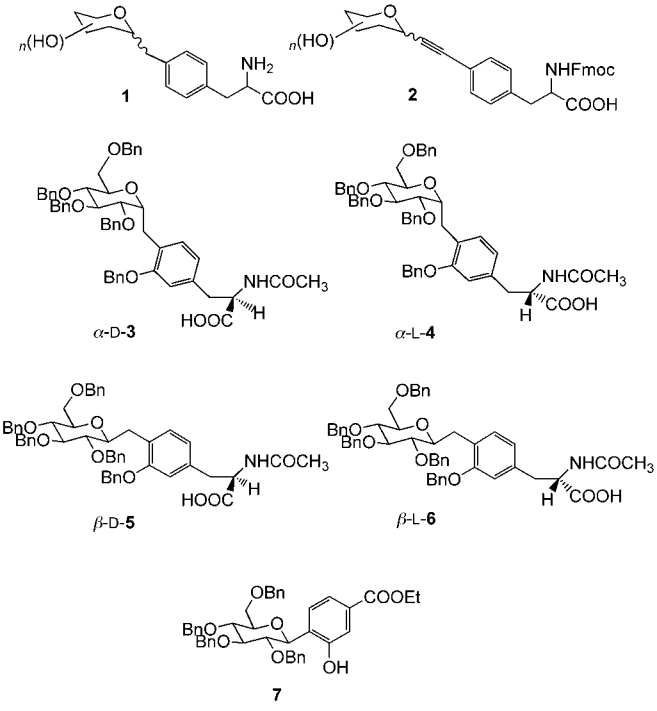
Keywords: amino acids · annulation · C-glycosides · glycopeptides

Introduction

It has been established that glycopeptides play a central role in biological recognition processes,[1] and there is increasing interest in their employment as pharmaceuticals. This approach is limited by the inherent instability of the O-glycosyl linkage in vivo: The lifetime of glycopeptide drugs could be increased by thwarting the enzymatic cleavage of the glycan from the peptide backbone. It has been observed that resistance to enzymatic hydrolysis could be achieved either by preparing non-natural O-glycosyl amino acids,[2] by substituting the glycosidic C–O bonds with C–C or C–S bonds,[3] or by replacing the sugar moiety with a cyclohexane ring which bears suitable hydroxylic functions.[4]

The syntheses of several anomeric C-glycosyl α-amino acids have been recently reviewed.[5] In the case of derivatives of the aromatic series, only one paper is devoted to the preparation of C-glycosyl tyrosines of the general structure type **1**, which possess a methylene group instead of the phenolic oxygen of tyrosine.[6] Prior to this work, a series of phenylalanine-containing C-glycosides of structure type **2**, which are based on a two-carbon alkyne-linkage, had been prepared.[7]

In this paper we report on the preparation of α- and β-C-glucosyl tyrosine analogues **3**–**6**, which have a hydroxyl function on the aromatic ring. The extra phenolic group is a consequence of the chemistry used to build the arene, but it can be conveniently exploited for further derivatisation of the substrate with a sugar moiety. We have devised a quite general route for the synthesis of derivative **3**–**6**, which could be easily applied to prepare other tyrosine analogues that have different glycan moieties, or to vary the spacer units between the sugar and the amino acid. As a matter of fact, for the formation of the functionalised aromatic ring we employed a benzoannulation procedure we had previously optimised,[8] and had already applied to the synthesis of C-glycoside **7**.[9]



Results and Discussion

Synthesis of the aromatic moiety: In the known examples of C-glycosyl tyrosine of structural formula **1** the aromatic

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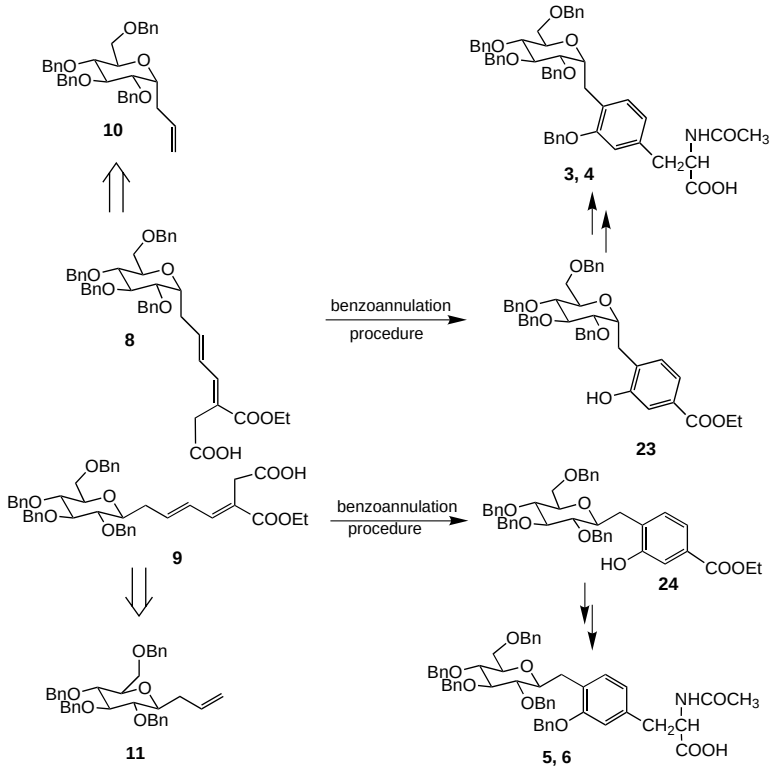
moiety was introduced with the formation of the crucial C-glycoside linkage by the addition of an organozinc species to a glucal derivative. The authors observed that the α/β selectivity associated with this C-glycosylation method dramatically depended on the nature of the sugar component. We preferred a different approach (Scheme 1), which is based on the formation of the aromatic ring after the establishment of the anomeric linkage, in order to optimise a general synthetic path to both α- and β-C-glycosyl amino acids. We took advantage of our experience in the reaction of 3-alkoxycarbonyl-3,5-hexadienoic acids with ethyl chloroformate in THF in the presence of triethylamine. This reaction gave 3-hydroxy-benzoate ester derivatives from a benzoannulation mechanism. The application of this synthetic scheme required the preparation of monoester monoacid derivatives **8** and **9**, as shown in Scheme 1. The α- and β-allyl glucosides **10** and **11**, which have a well-defined configuration at the anomeric carbon atom, were envisaged as suitable precursors of the key intermediates **8** and **9** (Scheme 1).

Two common reactions of sugar chemistry that allow high stereochemical control on the configuration of the anomeric carbon atom were employed (see Scheme 2). From the methodologies for the preparation of α-C-glycosides,[10] we chose the acid-catalysed addition reported by Giannis et al.[11] Treatment of acetyl derivative **12** with allyltrimethylsilane in acetonitrile in the presence of $BF_3 \cdot Et_2O$ at 4 °C afforded **10** with high stereoselectivity (>99%, ^{1}H NMR)[12] (Scheme 2). The reaction requires the presence of an acyl group at the anomeric carbon atom and is applicable to a variety of glycopyranosides and to disaccharides.[11]

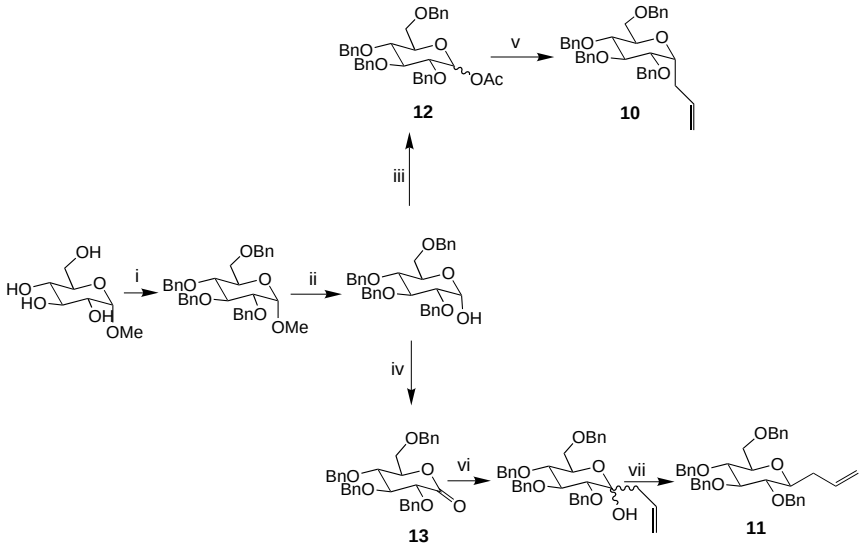
Several methods are available for the installation of carbon functionality to glycosides with exclusive equatorial selectivity.[10] We chose the approach of Kishi et al.,[13] which consists of a Grignard addition to a perbenzylated sugar lactone, and which is followed by deoxygenation of the resulting hemiketal. The reaction of allylmagnesium chloride with the lactone **13** in THF, which is followed by treatment with Et_3SiH, gave the β-allyl glucoside **11** with 99% stereoselectivity (^{1}H NMR)[12] (Scheme 2).

The derivatives **12** and **13** were prepared from methyl α-D-glucopyranoside according to the known routes,[14] as shown in Scheme 2.

Compounds **10** and **11** were manipulated separately through conventional organic reactions to provide the monoester and monoacid derivatives **8** and **9** (Scheme 3), respec-



Scheme 1. Synthetic procedure to derivatives **3**–**6**.



Scheme 2. i) Benzyl chloride, KOH, dioxane; ii) H_2SO_4, 4N acetic acid; iii) Ac_2O, pyridine; iv) CrO_3, H_2SO_4; v) allyltrimethylsilane, $BF_3 \cdot Et_2O$, acetonitrile, 4 °C; vi) allylmagnesium chloride; vii) Et_3SiH, $BF_3 \cdot Et_2O$.

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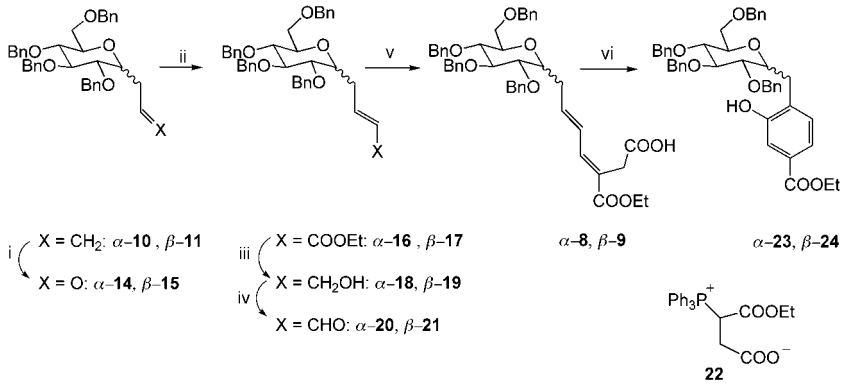


Scheme 3. i) ozone, CH_2Cl_2/MeOH 1:1 *v*/*v*, then PPH_3; ii) PPh_3=CHCOOEt, toluene; iii) DIBAL, toluene, −78 °C; iv) manganese(IV)oxide, CH_2Cl_2; v) betaine **22**, CH_2Cl_2; vi) ethyl chloroformate, Et_3N, THF.

tively. Ozonolysis of both C-allyl glucosides in methanol/methylene chloride (1:1 *v*/*v*) gave the aldehydes **14** and **15**, after the reaction was quenched with triphenylphosphine. Compounds **14** and **15** were condensed with (triphenyl-λ^5-phosphanylidene)-acetic ethyl ester to afford the unsaturated ethyl esters **16** and **17**. Reduction with diisobutyl aluminum hydride and oxidation of allylic alcohols **18** and **19** with manganese(IV) oxide in methylene chloride gave unsaturated aldehydes **20** and **21**, which were treated with betaine **22**[15] to introduce the last functionalised double bond and obtain the monoester monoacid derivatives **8** and **9**. The *E*-configuration was assigned to this new double bond on the basis of previous studies on the stereochemistry of the Wittig reaction of betaine **22** with aldehydes.[15b,c] This reaction gives 3-(*E*)-alkylidenesuccinic acid derivatives with high diastereoselectivity.

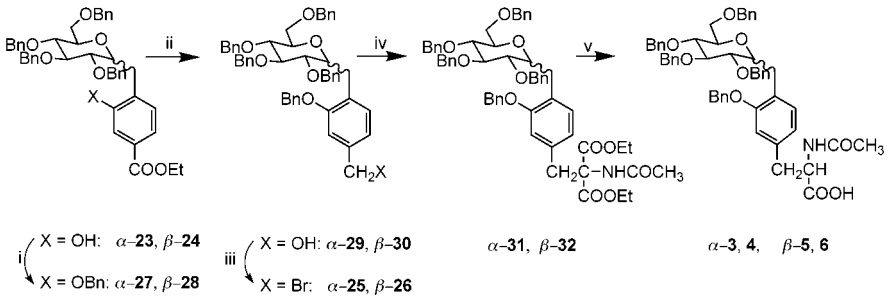
Compounds **8** and **9** rapidly underwent reaction with ethyl chloroformate in THF in the presence of triethylamine to provide the desired aromatic derivatives **23** and **24** in high yields.

Synthesis of the amino acid functionality: In the synthesis of **1**, the introduction of the amino acid moiety was achieved using the so-called Jackson method, involving a Pd^0-mediated coupling with an enantiomerically pure zinc reagent which already bears the amino acid function.[6] Amino acid derivatives in diastereoisomerically pure form were obtained, but we found that the efficiency of the transformation was extremely sensitive to reaction conditions. We chose a different approach based on the reaction of the bromo derivatives **25** and **26** with diethyl acetamido malonate[16] (Scheme 4). This implied a further step to separate the D and L stereoisomers of both the α and β series.

The phenolic group was protected as a benzyl ether by reaction of compounds **23** and **24** with potassium carbonate and benzyl bromide in acetone solution. These derivatives **27** and **28** were then submitted to lithium aluminum hydride reduction to afford alcohols **29** and **30**, which were immediately brominated with PPh_3 and NBS in order to provide compounds **25** and **26**, respectively. The reaction of **25** and **26** with diethyl acetamidomalonate in DMF solution with NaH as a base, proceeded rapidly and produced high yields. Treatment of **31** and **32** with sodium hydroxide in refluxing ethanol gave a 1:1 mixture of the two possible diastereoisomeric *N*-acetamido acids, both in the α-(**3** and **4**) and in β-series (**5** and **6**). Separation of α-D-**3** from its diastereoisomer α-L-**4** and of β-D-**5** from its diastereoisomer β-L-**6** was thus required.

Enzymatic deacylation: The two mixtures of α-D,L- and β-D,L-stereoisomeric N-acetamides were submitted separately to enzymic deacetylation at 25 °C in the presence of acylase I from *Aspergillus species*; the pH was maintained at 7.8 by addition of 0.02 M NaOH. The reactions were monitored by NMR spectroscopy. The signals of the methyl group of the acetamido moieties appeared at: CH_3CONH $\delta(\mathbf{3}) = 1.83$, $\delta(\mathbf{4}) = 1.86$ for the α-stereoisomers, $\delta(\mathbf{5}) = 1.80$, $\delta(\mathbf{6}) = 1.82$ for the β-stereoisomers. When no further evolution in the ratio of the two integrals was observed, the enzymatic reactions were stopped (48 h in both cases).

Acylase I is a widely applicable enzymatic catalyst for the kinetic resolution of unnatural and rare α-amino acids.[17] It promotes the hydrolysis of N-acetylated amino acids with L-configuration and accepts substrates that have a wide range of structure and functionality.[17] Thus, the two *N*-acetyl L-amino acids **4** and **6**, both with diastereomeric excess (*de*) >99% (^{1}H NMR), were recovered from the water phase after suitable treatment with acetic anhydride at pH 8 (see Experimental Section). The two unreacted *N*-acetyl D-amino acids **3** and **5** were found to contain 24 and 13%, respectively, of the corresponding L epimer. The stereoisomers **3**–**6** were converted into the corresponding methyl esters by diazomethane treatment for optical and spectroscopic characterisation.



Scheme 4. i) K_2CO_3, benzyl bromide, acetone; ii) $LiAlH_4$, THF; iii) PPh_3, NBS, THF; iv) diethyl acetamidomalonate, NaH, DMF; v) NaOH, ethanol.

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Conclusion

A general method for the synthesis of C-glycosyl amino acid was developed, and here described for the preparation of stereoisomerically pure tyrosine analogues α-L-**4** and β-L-**6** in reasonable overall yields from allyl derivatives **10** and **11**.

The application of the annulation procedure allowed us to build the aromatic ring, after the anomeric linkage had been created under strict stereochemical control through well-established reactions of carbohydrate chemistry. Thus, the glycosyl moiety can be easily modulated and both α- and β-derivatives can be prepared as single diastereoisomers.

The method is very flexible and can be applied to the synthesis of other C-glycosyl amino acids with different spacers between the sugar moiety and the aromatic amino acid. For example, it is possible to increase or decrease the number of the methylene units that separate the sugar moiety from the pseudo-tyrosine fragment. The key step is the preparation of the unsaturated aldehydes to be submitted to condensation with the betaine **22**, in order to afford the monoester monoacid substrates for benzoannulation procedure. Indeed, the application of the same sequence to derivative **7** allows us to successfully prepare β-C-glucosyl-3-hydroxyphenylalanine.

The mechanism of the cyclisation route implies the introduction of a hydroxyl group onto the aromatic ring. This functional group can be exploited to derivatise the C-glucosyl amino acid with other sugar moieties through a C–O anomeric linkage, in order to investigate the effects of this double glycosylation. The hydroxylic function can also be removed, after deprotection by hydrogenolysis, by reduction of the *O*-tetrazole derivative,[8] in order to provide carbon-linked analogue of natural glycoamino acids.

Experimental Section

General remarks: Acylase I from *Aspergillus species* (Sigma) was employed in this work. ^{1}H NMR spectra were recorded on a Bruker AC-250 (250 MHz, ^{1}H), or on a Bruker ARX 400 (400 MHz, ^{1}H), or on a Bruker Avance 500 (500 MHz, ^{1}H) spectrometer. A pH-Stat 718 Stat Titrino Metrohm was used to perform enzymatic deacylation. Optical rotations were measured on a Dr. Kernchen Propol digital automatic polarimeter. Microanalyses were determined on a Analyzer 1106 Carlo Erba. All the chromatographic separations were carried out on silica gel columns. In the NMR assignment, the aromatic ring, which is built by benzannulation, is referred to as benzene ring A. The numbering of its carbon atoms used in the NMR description is as specified in the name of the compound itself.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose was prepared from commercial starting material, methyl α-D-glucopyranoside, by following the procedure in ref. [14a]. Acetylation of this derivative in acetic anhydride and pyridine gave **12**.[14b] 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose was oxidised to 2,3,4,6-tetra-*O*-benzyl-D-glucono-δ-lactone (**13**) by treatment with Jones reagent in acetone solution, rather than through the use of DMSO in acetic anhydride as reported in ref. [14c].

1-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-yl)-2-propene (10): Allyltrimethylsilane (19.6 g, 0.172 mol) and boron trifluoride etherate (44 mL, 0.344 mol) were added successively to a solution of derivative **12** (50.0 g, 0.086 mol) in acetonitrile (300 mL) under nitrogen atmosphere at 4 °C. After 24 h at 4 °C, the reaction mixture was poured into a saturated solution of sodium hydrogen carbonate and extracted with methylene chloride. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure to give a residue which was chromatographed, eluted with hexane/ethyl acetate 7:3 *v/v*. Compound **10** was isolated as a white solid (43.16 g, 89 %). M.p. 60 °C (lit.[18] 64–65 °C), $[\alpha]_D^{20} = 34.2$ ($c = 1.02$ in $CHCl_3$);[18] $[\alpha]_D = 36.5$, $c = 2.19$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.5-7.0$ (m, 20H; $4C_6H_5$), 5.82 (m, 1H; C*H*=CH_2), 5.11 (dd, $J = 13$, 1.7 Hz, 1H; CH=C*H*H), 5.06 (dd, $J = 6$, 1.7 Hz, 1H; CH=C*H*H), 5.00–4.40 (m, 8H; $4CH_2$Ph), 4.13 (m, 1H; H-$C_{anomeric}$), 3.90–3.50 (m, 6H; sugar moiety), 2.47 (m, 2H; CH_2CH=CH_2); elemental analysis calcd (%) for $C_{37}H_{40}O_5$ (564.7): C 78.69, H 7.14; found: C 78.64, H 7.20.

1-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-yl)-2-propene (11): Lactone **13** was azeotroped with toluene prior to use. A 2 M solution of allylmagnesium chloride in THF (70 mL, 0.139 mol) was added to a solution of lactone **13** (50.0 g, 0.093 mol) in THF (300 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 2 h, treated with a 5 % NH_4Cl solution, and extracted with ethyl acetate. The residue, which consisted mainly of 1-hydroxy-1-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2-propene, (49.1 g, 91 %) was azeotroped with toluene and used as obtained.

Et_3SiH (29.3 g, 0.252 mol) and $BF_3 \cdot Et_2O$ (21.3 mL) were added to a solution of 1-hydroxy-1-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2-propene (49.1 g, 0.084 mol) in methylene chloride (400 mL) at −78 °C. The reaction was stirred at −78 °C for 12 h. Saturated aqueous $NaHCO_3$ was added, and the reaction mixture extracted with CH_2Cl_2. The solid residue was recrystallised from hexane/ethyl acetate (95:5 *v/v*) to give derivative **11** (37.1 g, 78 %). M.p. 85 °C (lit.: 91–92 °C)[19]; $[\alpha]_D^{20} = 15.6$ ($c = 1.11$ in $CHCl_3$) (lit.: $[\alpha]_{20}^{D} = 13.5$)[19]; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.4-7.0$ (m, 20H; $4C_6H_5$), 5.94 (m, 1H; C*H*=CH_2), 5.20–5.00 (m, 2H; CH=CH_2), 5.00–4.50 (m, 8H; $4CH_2$Ph), 3.80–3.50 (m, 4H; sugar moiety), 3.41 (ddd, $J = 9.4$, 4.1, 2.2 Hz, 1H; sugar moiety), 3.34 (m, 2H; sugar moiety), 2.60 (m, 1H; C*H*HCH=CH_2), 2.32 (m, 1H; C*H*HCH=CH_2); elemental analysis calcd (%) for $C_{37}H_{40}O_5$ (564.7): C 78.69, H 7.14; found: C 78.73, H 7.22.

4-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-yl)-but-2-enoic ethyl ester (16):[20] A solution of allyl derivative **10** (43.0g, 0.076 mol), in CH_2Cl_2/MeOH (1:1 *v/v*, 150 mL) was treated with ozone at −78 °C. The reaction mixture was quenched with triphenylphosphine, warmed to room temperature, and concentrated. The resulting aldehyde **14** was used immediately. A solution of **14** and of (triphenyl-λ^5-phosphanylidene)-acetic acid ethyl ester (40 g, 0.114 mol) in toluene (100 mL) was heated under reflux for 2 h. The residue was chromatographed and eluted with hexane/ethyl acetate 8:2 *v/v* to afford ester derivative **16** (24.3 g, 50 %). $[\alpha]_D^{20} = 46.6$ ($c = 1.20$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.4-7.1$ (m, 20H; $4C_6H_5$), 6.95 (dt, $J = 6.4$, 16.0 Hz, 1H; CH_2C*H*=CH), 5.90 (d, $J = 16.0$ Hz, 1H; CH_2CH=C*H*), 5.00–4.40 (m, 8H; $4CH_2$Ph), 4.15 (m + q, $J = 7.1$ Hz, 3H; H-$C_{anomeric}$, $COOCH_2CH_3$), 3.80–3.50 (m, 6H; sugar moiety), 2.62 (m, 2H; CH_2CH=CH_2), 1.26 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{40}H_{44}O_7$ (636.8): C 75.45, H 6.96; found: C 75.68, H 7.08.

4-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-yl)-but-2-enoic ethyl ester (17): The same procedure was used to convert derivative **11** (37 g, 0.066 mol) via aldehyde **15** into ester **17** (22.9 g, 55 %). $[\alpha]_D^{20} = -31.3$ ($c = 1.15$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.4-7.1$ (m, 20H; $4C_6H_5$), 6.97 (dt, $J = 7.4$, 15.7 Hz, 1H; CH_2C*H*=CH), 5.82 (d, $J = 15.7$ Hz, 1H; CH_2CH=C*H*), 4.90–4.75 (m, 4H; $2CH_2$Ph), 4.65–4.45 (m, 4H; $2CH_2$Ph), 4.12 (q, $J = 7$ Hz, 2H; $COOCH_2$), 3.70–3.55 (m, 4H, sugar moiety), 3.40–3.20 (m, 3H; sugar moiety); 2.63 (m, 1H; C*H*HCH=CH_2), 2.35 (m, 1H; C*H*HCH=CH_2), 1.26 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{40}H_{44}O_7$ (636.7): C 75.45, H, 6.96; found: C 75.53, H 7.11.

4-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-yl)-but-2-en-1-ol (18):[20] A 1.5 M diisobutyl aluminum hydride in toluene (76 mL, 0.114 mol) was added dropwise to a solution of the ester, **16** (24.0 g, 0.038 mol), in toluene (200 mL) at −78 °C. The reaction was warmed to 25 °C, treated with water, filtered through a Celite cake, and extracted with ethyl acetate. The residue was chromatographed, and eluted with hexane/ethyl acetate 6:4 *v/v* to give the allylic alcohol **18** (19.4 g, 86 %). M.p. 73 °C; $[\alpha]_D^{20} = 42.2$ ($c = 1.26$ in $CHCl_3$) (lit.: $[\alpha]_{20}^{D} = 44.2$, $c = 1.56$ in $CHCl_3$)[20]; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta =$ 7.4–7.0 (m, 20H; $4C_6H_5$), 5.70 (m, 2H; C*H*=C*H*), 5.00–4.35 (m, 8H; $4CH_2$Ph), 4.15–4.00 (m+d, $J = 5$ Hz, 3H; H-$C_{anomeric}$, CH_2OH), 3.90–3.45 (m, 6H; sugar moiety), 2.47 (m, 2H; CH_2CH=CH); elemental analysis calcd (%) for $C_{38}H_{42}O_6$ (594.7): C 76.74, H 7.12; found: C 76.61, H 7.18.

4-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-yl)-but-2-en-1-ol (19): The same procedure was used on compound **17** (22.0 g, 0.034 mol) to give allylic alcohol **19** (18.1 g, 88%): $[\alpha]_D^{20} = 0.4$ ($c = 1.38$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.4-7.1$ (m, 20H; $4C_6H_5$), 5.73 (m, 2H; C*H*=C*H*), 4.95–4.75 (m, 4H; $2CH_2$Ph), 4.70–4.50 (m, 4H; $2CH_2$Ph), 4.04 (d, $J = 6.4$ Hz, 2H; CH_2OH), 3.80–3.50 (m, 4H; sugar moiety), 3.40 (ddd, $J = 9.8$, 4.4, 2.2 Hz, 1H; H-C_5 sugar moiety), 3.31 (m, 2H; sugar moiety), 2.56 (m, 1H; C*H*H CH=CH), 2.28 (m, 1H; C*H*HCH=CH); elemental analysis calcd (%) for $C_{38}H_{42}O_6$ (594.7): C 76.74, H 7.12; found: C 76.85, H 7.05.

7-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-yl)-3-ethoxycarbonyl-hepta-3,5-dienoic acid (8): A mixture of the allylic alcohol **18** (19.0 g, 0.032 mol) and manganese(IV)oxide (1.5 equiv) in methylene chloride (100 mL) was heated under reflux for 3 h. The reaction mixture was filtered, and the dissolved aldehyde **20** was used immediately. Betaine **22**[15] (19.7 g, 0.048 mol) was added directly to the aldehyde solution, and the reaction mixture was heated under reflux for 3 h, concentrated under reduced pressure, and chromatographed. Elution with hexane/ethyl acetate 1:1 *v*/*v* gave the unsaturated acid **8** (14.7 g, 64%). $[\alpha]_D^{20} = 50.3$ ($c = 1.18$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.6-6.9$ (m, 21H; $4C_6H_5$, CH=CHC*H*=), 6.36 (dd, $J = 11$, 15 Hz, 1H; CH=C*H*CH=), 6.18 (dt, $J = 6$, 15 Hz, 1H; C*H*=CHCH=), 5.00–4.40 (m, 8H; $4CH_2$Ph), 4.17 (m, 3H; H-$C_{anomeric}$, $COOCH_2CH_3$), 3.80–3.25 (m, 8H; 6 × sugar moiety, CH_2COOH), 2.61 (m, 2H; CH_2-$C_{anomeric}$), 1.29 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{44}H_{48}O_9$ (720.8): C 73.31, H 6.71; found: C 73.41, H 6.87.

7-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-yl)-3-ethoxycarbonyl-hepta-3,5-dienoic acid (9): The same procedure was used to convert the allylic alcohol **19** (18 g, 0.030 mol) into **9**, an unsaturated acid (13.3 g, 61%). $[\alpha]_D^{20} = -2.3$ ($c = 0.44$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.6-6.9$ (m, 21H; $4C_6H_5$, vinylic), 6.30 (m, 2H; vinylic), 5.00–4.50 (m, 8H; $4CH_2$Ph), 4.24 (m, 2H; $COOCH_2CH_3$), 3.80–3.25 (m, 9H; 7 × sugar moiety, CH_2COOH), 2.70 (m, 1H; C*H*H-$C_{anomeric}$), 2.42 (m, 1H; C*H*H-$C_{anomeric}$), 1.31 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{44}H_{48}O_9$ (720.8): C 73.31, H 6.71; found: C 73.21, H 6.64.

4-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)-3-hydroxybenzoic ethyl ester (23): Ethyl chloroformate (2.47 g, 0.023 mol) was dropped into a solution of the unsaturated acid **8** (14.0 g, 0.019 mol) in THF (35 mL). Et_3N (2.32 g, 0.023 mol) was added, and the temperature maintained below 20 °C. The reaction mixture was stirred at room temperature for 30 min, poured into diluted HCl, and extracted with ethyl acetate. The residue was chromatographed and eluted with hexane/ethyl acetate 9:1 *v*/*v* to give compound **23** (9.20 g, 69%). M.p. 118 °C; $[\alpha]_D^{20} = 49.3$ ($c = 1.03$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.50$ (m, 2H; benzene ring A), 7.4–7.1 (m, 20H; $4C_6H_5$), 7.09 (d, $J = 8$ Hz,1H; H-C_5 of benzene ring A), 7.05 (br s, 1H; OH), 4.90–4.40 (m, 8H; $4CH_2$Ph), 4.34 (q, $J = 7$ Hz, 2H; $COOCH_2CH_3$), 4.27 (m, 1H; H-$C_{anomeric}$), 4.02 (dt, $J = 8.1$, 3.5 Hz, 1H; H-C_5 of the sugar moiety), 3.85 (t, $J = 8.1$ Hz, 1H; sugar moiety), 3.71 (dd, $J = 8.1$, 5.1 Hz, 1H; *H*-C_2 of the sugar moiety), 3.63 (d, $J = 3.5$ Hz, 2H; CH_2OBz of the sugar moiety), 3.58 (t, $J = 8.1$ Hz, 1H; sugar moiety), 3.10 (dd, $J = 9.7$, 14.7 Hz, 1H, C*H*H-$C_{anomeric}$), 2.97 (dd, $J = 3$, 14.7 Hz, 1H; C*H*H-$C_{anomeric}$), 1.37 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{44}H_{46}O_8$ (702.8): C 75.19, H 6.60; found: C 75.31, H 6.71.

4-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-ylmethyl)-3-hydroxybenzoic ethyl ester (24): The same procedure was used to convert the unsaturated acid **9** (13.0 g, 0.018 mol) into its aromatic derivative **24** (8.23 g, 65%). M.p. 108 °C; $[\alpha]_D^{20} = 12.2$ ($c = 0.98$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.88$ (s, 1H; OH), 7.60 (d, $J = 2$ Hz, 1H; H-C_2 of benzene ring A), 7.49 (dd, $J = 8$, 2 Hz, 1H; H-C_6 of benzene ring A), 7.4–7.1 (m, 20H; $4C_6H_5$), 7.02 (d, $J = 8$ Hz, 1H; H-C_5 of benzene ring A), 5.00–4.75 (m, 4H; $2CH_2$Ph), 4.65–4.45 (m, 4H; $2CH_2$Ph), 4.36 (q, $J = 7$ Hz, 2H; $COOCH_2CH_3$), 3.75–3.55 (m, 5H; sugar moiety), 3.48 (m, 1H; sugar moiety), 3.33 (t, $J = 8.5$ Hz, 1H; sugar moiety), 3.09 (dd, $J = 2.7$, 14.7 Hz, 1H; C*H*H-$C_{anomeric}$), 2.92 (dd, $J = 7.8$, 14.7 Hz, 1H; C*H*H-$C_{anomeric}$), 1.38 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{44}H_{46}O_8$ (702.8): C 75.19, H 6.60; found: C 75.09, H 6.48.

4-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)-3-benzyloxybenzoic ethyl ester (27): Benzyl bromide (2.52 g, 0.020 mol) was added to a mixture of derivative **23** (9.20 g, 0.013 mol) and potassium carbonate (2.71 g, 0.020 mol) in acetone (50 mL). The reaction mixture was stirred for 12 h, diluted with water and extracted with ethyl acetate. The residue was crystallised from hexane/ethyl acetate 9:1, to afford the benzyl ether-protected derivative **27** (8.75 g, 85%). M.p. 82 °C; $[\alpha]_D^{20} = 71$ ($c = 1.01$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.61$ (d, $J = 1.5$ Hz, 1H; H-C_2 of benzene ring A), 7.55 (dd, $J = 8$, 1.5 Hz, 1H; H-C_6 of benzene ring A), 7.45–7.10 (m, 26H; $5C_6H_5$, H-C_5 of benzene ring A), 5.10 (s, 2H; PhCH_2O-C_3 of benzene ring A), 4.92 (d, $J = 11$ Hz, 1H; C*H*HPh), 4.81 (d, $J = 11$ Hz, 1H; C*H*HPh), 4.77 (d, $J = 11$ Hz, 1H; C*H*HPh), 4.56 (m, 1H; H-$C_{anomeric}$), 4.53–4.30 (m, 7H; 5C*H*HPh, $COOCH_2CH_3$), 3.85 (m, 2H; sugar moiety), 3.75 (dd, $J = 6$, 9 Hz, 1H; *H*-C_2 of the sugar moiety), 3.60–3.45 (m, 3H; sugar moiety), 3.28 (dd, $J = 3.2$, 14.5 Hz, 1H; C*H*H-$C_{anomeric}$), 2.99 (dd, $J = 11$, 14.5 Hz, 1H; C*H*H-$C_{anomeric}$), 1.39 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{51}H_{52}O_8$ (792.9): C 77.25, H 6.61; found: C 77.46, H 6.75.

4-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-ylmethyl)-3-benzyloxybenzoic ethyl ester (28): The same procedure was used to transform derivative **24** (8.0 g, 0.011 mol) into compound **28** (7.56 g, 87%). M.p. 107 °C; $[\alpha]_D^{20} = -2.2$ ($c = 1.96$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.7-7.1$ (m, 28H; 3 × benzene ring A, $5C_6H_5$), 5.13 (m, 2H; PhCH_2O-C_3 of benzene ring A), 5.0–4.40 (m, 8H; $4CH_2$Ph), 4.34 (q, $J = 7$ Hz, 2H; $COOCH_2CH_3$), 3.80–3.30 (m, 8H; 7 × sugar moiety, C*H*H-$C_{anomeric}$), 2.72 (dd, $J = 9.5$, 14 Hz, 1H; C*H*H-$C_{anomeric}$), 1.39 (t, $J = 7$ Hz, 3H; $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{51}H_{52}O_8$ (792.9): C 77.25, H 6.61; found: C 77.08, H 6.43.

1-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)-2-benzyloxy-4-hydroxymethylbenzene (29): A solution of ester **27** (8.50 g, 0.011 mol) in THF (10 mL) was added dropwise to a suspension of $LiAlH_4$ (1.25 g, 0.033 mol) in THF (100 mL), and the temperature was maintained below 20 °C. The reaction mixture was stirred at room temperature for 1 h. The usual work-up afforded a solid residue, which was recrystallised from hexane/ethyl acetate 8:2 *v*/*v* to give the alcohol **29** (7.34 g, 89%). M.p. 102 °C; $[\alpha]_D^{20} = 63.3$ ($c = 1.12$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.7-7.1$ (m, 26H; 1 × benzene ring A, $5C_6H_5$), 6.95 (d, $J = 1.15$ Hz, 1H; H-C_3 of benzene ring A), 6.81 (dd, $J = 1.15$, 8 Hz, 1H; H-C_5 of benzene ring A), 5.06 (s, 2H; PhCH_2O-C_2 of benzene ring A), 5.0–4.30 (m, 11H; $4CH_2$Ph, H-$C_{anomeric}$, CH_2OH), 3.90–3.45 (m, 6H; sugar moiety), 3.25 (dd, $J = 3$, 14.5 Hz, 1H; C*H*H-$C_{anomeric}$), 2.92 (dd, $J = 12$, 14.5 Hz, 1H; C*H*H-$C_{anomeric}$); elemental analysis calcd (%) for $C_{49}H_{50}O_7$ (750.9): C 78.37, H 6.71. found: C 78.19, H 6.56.

1-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-ylmethyl)-2-benzyloxy-4-hydroxymethyl benzene (30): The same procedure was used to convert the ester derivative **28** (7.0 g, 8.84 mmol) to the alcohol **30** (6.03 g, 91%). M.p. 73 °C; $[\alpha]_D^{20} = -2.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.5-7.1$ (m, 26H; 1 × benzene ring A, $5C_6H_5$), 6.91 (d, $J = 1.15$ Hz, 1H; H-C_3 of benzene ring A), 6.81 (dd, $J = 1.15$, 8 Hz, 1H; H-C_5 of benzene ring A), 5.10 (s, 2H; PhCH_2O-C_2 of benzene ring A), 4.90–4.35 (m, 10H; $4CH_2$Ph, CH_2OH), 3.80–3.25 (m, 8H; 7 × sugar moiety, C*H*H-$C_{anomeric}$), 2.67 (dd, $J = 9.7$, 14.6 Hz, 1H; C*H*H-$C_{anomeric}$); elemental analysis calcd (%) for $C_{49}H_{50}O_7$ (750.9): C 78.37, H 6.71; found: C 78.49, H 6.87.

1-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)-2-benzyloxy-4-bromomethyl benzene (25): NBS (2.49 g, 0.014 mol) was added to a solution of alcohol **29** (7.0 g, 9.33 mmol) and PPh_3 (3.67 g, 0.014 mol) in THF (50 mL), while the temperature was kept below 30 °C. The reaction mixture was stirred at room temperature for 2 h and concentrated. Bromomethyl derivative **25** (6.22 g, 82%) was recovered by chromatography and eluted with hexane/ethyl acetate 8:2 *v*/*v*. M.p. 93 °C; $[\alpha]_D^{20} = 47.4$ ($c = 1.05$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.4-7.1$ (m, 26H; 1 × benzene ring A, $5C_6H_5$), 6.94 (d, $J = 1.15$ Hz, 1H; H-C_3 of benzene ring A), 6.84 (dd, $J = 1.15$, 8 Hz, 1H; H-C_5 of benzene ring A), 5.05 (s, 2H, PhCH_2O-C_2 of benzene ring A), 4.92 (d, $J = 11$ Hz, 1H; C*H*HPh), 4.81 (d, $J = 11$ Hz, 1H; C*H*HPh), 4.76 (d, $J = 11$ Hz, 1H, C*H*HPh), 4.60–4.30 (m, 8H; 5C*H*HPh, *H*-$C_{anomeric}$, CH_2Br), 3.85 (m, 2H; sugar moiety), 3.74 (m, 1H; sugar moiety), 3.85 (m, 3H; sugar moiety), 3.23 (dd, $J = 3.4$, 14.7 Hz, 1H; C*H*H-$C_{anomeric}$), 2.93 (dd, $J = 12$, 14.7 Hz, 1H; C*H*H-$C_{anomeric}$); elemental analysis calcd (%) for $C_{49}H_{49}BrO_6$ (813.8): C 73.32, H 6.07, Br 9.82; found: C 73.44, H 6.19, Br 9.67.

1-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranos-1-ylmethyl)-2-benzyloxy-4-bromomethyl benzene (26): According to the same procedure alcohol **30** (5.90 g, 7.87 mmol) was transformed into bromo derivative **26** (5.05 g, 79%). M.p. 110 °C; $[\alpha]_D^{20} = -7.1$ ($c = 0.88$ in $CHCl_3$); ^{1}H NMR (250 MHz,

0947-6539/02/0808-1876 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 8

$CDCl_3$, 25 °C, TMS): $\delta = 7.5-7.1$ (m, 26H; 1 × benzene ring A, 5 C_6H_5), 6.98 (m, 2H; benzene ring A), 5.09 (s, 2H; PhCH_2O-C_2 of benzene ring A), 4.90–4.40 (m, 10H, 4 CH_2Ph, CH_2Br), 3.80–3.30 (m, 8H; 7 × sugar moiety, CHH-$C_{anomeric}$), 2.66 (dd, $J = 9.4$, 14.5 Hz, 1H, CHH-$C_{anomeric}$); elemental analysis calcd (%) for $C_{49}H_{49}BrO_6$ (813.8): C 73.32; H 6.07, Br 9.82; found: C 73.56, H 6.12, Br 9.98.

2-Acetamido-2-[3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)benzyl] malonic diethyl ester (31): Diethyl acetamidomalonate (2.39 g, 0.011 mol) was added to a suspension of NaH (0.42 g, 60% in mineral oil, 0.011 mol) in DMF (20 mL). After 20 min, the bromo derivative **25** (6.0 g, 7.38 mmol) in DMF (5 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, poured into water and extracted with ethyl acetate. The residue was chromatographed and eluted with hexane/ethyl acetate 8:2 *v*/*v*, and pure derivative **31** (4.83 g, 69%) was obtained after recrystallisation from hexane/ethyl acetate 8:2 *v*/*v*. M.p. 61 °C; $[\alpha]_D^{20} = 37.6$ ($c = 1.03$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.4-7.0$ (m, 25H; 5 C_6H_5), 7.05 (d, $J = 7.5$ Hz, 1H, H-C_5 of benzene ring A), 6.55 (d, $J = 1.15$ Hz, 1H; H-C_2 of benzene ring A), 6.52 (s, 1H; NH), 6.50 (dd, $J = 1.15$, 7.5 Hz, 1H; H-C_6 of benzene ring A), 4.98 (s, 2H; PhCH_2O-C_3 of benzene ring A), 4.93 (d, $J = 11$ Hz, 1H; CHHPh), 4.81 (d, $J = 11$ Hz, 1H; CHHPh), 4.77 (d, $J = 11$ Hz, 1H; CHHPh), 4.55–4.30 (m, 10H; H-$C_{anomeric}$, 5 CHHPh, 2 $COOCH_2CH_3$), 3.85 (m, 2H; sugar moiety), 3.75 (dd, $J = 6$, 9.5 Hz, 1H; sugar moiety), 3.70–3.60 (m, 4H; 2 × sugar moiety, CH_2CNH), 3.49 (dd, $J = 2$, 11 Hz, 1H; sugar moiety), 3.21 (dd, $J = 3$, 14.7 Hz, 1H; CHH-$C_{anomeric}$), 2.90 (dd, $J = 11.6$, 14.7 Hz, 1H; CHH-$C_{anomeric}$), 1.92 (s, 3H; NHCOCH_3), 1.30–1.25 (m, 6H; 2 $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{58}H_{63}NO_{11}$ (950.1): C 73.32, H 6.68, N 1.47; found: C 73,47, H 6.56, N 1.23.

2-Acetamido-2-[3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-ylmethyl)benzyl] malonic diethyl ester (32): The same procedure was used to convert derivative **26** (4.90 g, 6.03 mmol) into compound **32** (3.72 g, 65%). M.p. 76–77 °C; $[\alpha]_D^{20} = -4.3$ ($c = 1.07$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.35-7.1$ (m, 26H; 5 C_6H_5, 1 × benzene ring A), 6.44 (m, 3H; 2 × benzene ring A, NH), 4.94 (m, 2H; PhCH_2O-C_3 of benzene ring A), 4.81 (m, 3H; 3 CHHPh), 4.75 (d, $J = 11$ Hz, 1H; CHHPh), 4.62 (d, $J = 11$ Hz, 1H; CHHPh), 4.52 (d, $J = 11$ Hz, 1H; CHHPh), 4.45 (d, $J = 11$ Hz, 1H; CHHPh), 4.37 (d, $J = 11$ Hz, 1H; CHHPh), 4.0–4.25 (m, 4H, 2 $COOCH_2CH_3$), 3.70–3.25 (m, 10H; 7 × sugar moiety, CH_2CNH, CHH-$C_{anomeric}$), 2.57 (dd, $J = 9.8$, 15 Hz, 1H; CHH-$C_{anomeric}$), 1.83 (s, 3H; NHCOCH_3), 1.25–1.15 (m, 6H; 2 $COOCH_2CH_3$); elemental analysis calcd (%) for $C_{58}H_{63}NO_{11}$ (950.1): C 73.32, H 6.68, N 1.47; found: C 73,25, H 6.79, N 1.59.

***N*-Acetamido-3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)-D,L-phenylalanine (3, 4)**: A mixure of derivative **31** (4.60 g, 4.85 mmol) and NaOH (0.290 g, 7.28 mmol) in ethanol (35 mL) was heated under reflux for 5 h. After the usual work-up, a 1:1 mixture (^{1}H NMR of the corresponding methyl ester) of diastereoisomeric derivatives **3** and **4** was recovered (3.21 g, 78%).

***N*-Acetamido-3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-ylmethyl)-D,L-phenylalanine (5, 6)**: The same procedure gave derivative **32** (2.20 g, 2.23 mmol) in a 1:1 mixture (^{1}H NMR of the corresponding methyl ester) of diastereoisomeric derivatives **5** and **6** (1.48 g, 75%).

***N*-Acetamido-3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)-D-phenylalanine (3)** and ***N*-acetamido-3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranos-1-ylmethyl)-L-phenylalanine (4)**: The enzyme, acylase I, (500 mg) was added to a suspension of the 1:1 mixture of derivatives **3** and **4** (3.0 g, 3.53 mmol) in water/isopropanol 6:1 (35 mL). The pH was kept at 7.8 by addition of NaOH (0.02 M) by a pH-Stat. The reaction mixture was stirred at room temperature for 48 h, and monitored by ^{1}H NMR spectroscopy. Samples of the reaction mixture were acidified, extracted with ethyl acetate, treated with diazomethane in Et_2O, and concentrated under reduced pressure. The signals of the methyl group of the acetamido moiety were monitored: CH_3CONH δ(**3**) = 1.83, δ(**4**) = 1.86. When no further evolution was observed, the mixture was acidified by addition of 1N HCl, and filtered. The filtrate was extracted with ethyl acetate. The organic phase afforded α-D-**3** (1.04 g, 35%) with 24% of the α-L-**4** diastereoisomer (^{1}H NMR of the corresponding methyl ester). M.p. 123 °C; elemental analysis calcd (%) for $C_{53}H_{55}NO_9$ (850.0): C 74.89, H 6.52, N 1.65; found: C 74.57, H 6.78, N 1.33. Treatment with diazomethane in CH_2Cl_2/Et_2O converted derivative **3** (*de* 52%) into the corresponding methyl ester for spectroscopic characterisation. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.35-7.05$ (m, 25H; 5 C_6H_5), 6.99 (d, $J = 7.6$ Hz, 1H; H-C_5 of benzene ring A), 6.56 (d, $J = 1.2$ Hz, 1H; H-C_2 of benzene ring A), 6.49 (dd, $J = 1.2$, 7.6 Hz, 1H; H-C_6 of benzene ring A), 5.73 (d, $J = 7.5$ Hz, 1H; NH), 4.95 (s, 2H; PhCH_2O-C_3 of benzene ring A), 4.86 (d, $J = 11$ Hz, 1H; CHHPh), 4.80–4.60 (m, 3H; 2 CHHPh, CHNH), 4.50–4.25 (m, 6H; 5 CHHPh, H-$C_{anomeric}$), 3.78 (m, 2H; sugar moiety), 3.68 (dd, $J = 6$, 9.4 Hz, 1H; sugar moiety), 3.61 (s, 3H; $COOCH_3$), 3.54 (m, 2H; sugar moiety), 3.44 (m, 1H; sugar moiety), 3.15 (dd, $J = 3$, 14.7 Hz, 1H; CHH-$C_{anomeric}$), 2.99 (m, 2H; CH_2CHNH), 2.84 (dd, $J = 11.6$, 14.7 Hz, 1H; CHH-$C_{anomeric}$), 1.83 (s, 3H, NHCOCH_3).

The aqueous phase was concentrated in vacuo and the residue was acetylated according to a modified Schotten–Baumann procedure. It was dissolved in water at pH 8 (KOH), treated with an excess of acetic anhydride. After 24 h the reaction mixture was concentrated, diluted with water, and extracted with ethyl acetate. The residue was recrystallised from isopropanol, to afford the diastereoisomerically pure (*de* > 99% from ^{1}H NMR spectrum of the corresponding methyl ester) derivative α-L-**4** (0.970 g, 32%). M.p. 141 °C; elemental analysis calcd (%) for $C_{53}H_{55}NO_9$ (850.0): C 74.89, H 6.52, N 1.65; found: C 74.70, H 6.88, N 1.98.

Treatment with diazomethane in CH_2Cl_2/Et_2O converted derivative **4** into the corresponding methyl ester for optical and spectroscopic characterisation: $[\alpha]_D = 53.6$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.35-7.0$ (m, 25H; 5 C_6H_5), 6.99 (d, $J = 7.6$ Hz, 1H; H-C_5 of benzene ring A), 6.56 (d, $J = 1.2$ Hz, 1H; H-C_2 of benzene ring A), 6.49 (dd, $J = 1.2$, 7.6 Hz, 1H; H-C_6 of benzene ring A), 5.75 (d, $J = 7.5$ Hz, 1H; NH), 4.94 (s, 2H; PhCH_2O-C_3 of benzene ring A), 4.86 (d, $J = 11$ Hz, 1H; CHHPh), 4.80–4.60 (m, 3H; 2 CHHPh, CHNH), 4.50–4.25 (m, 6H; 5 CHHPh, H-$C_{anomeric}$), 3.78 (m, 2H; sugar moiety), 3.68 (dd, $J = 6$, 9.4 Hz, 1H; sugar moiety), 3.60 (s, 3H, $COOCH_3$), 3.53 (m, 2H, sugar moiety), 3.44 (m, 1H; sugar moiety), 3.15 (dd, $J = 3$, 14.7 Hz, 1H; CHH$C_{anomeric}$), 2.99 (m, 2H; CH_2CHNH), 2.84 (dd, $J = 11.6$, 14.3 Hz, 1H; CHH-$C_{anomeric}$), 1.86 (s, 3H; NHCOCH_3).

***N*-Acetamido-3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-ylmethyl)-D-phenylalanine (5)** and ***N*-acetamido-3-benzyloxy-4-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranos-1-ylmetyl)-L-phenylalanine (6)**: The same procedure was used to produce diastereoisomerically pure (*de* > 99%) β-L-**6** (0.485 g, 36%), after recrystallisation from isopropanol, and the derivative β-D-**5** (0.512 g, 38%) with impurity of 13% of diastereoisomer **6** from the 1:1 mixture of derivatives **5** and **6** (1.35 g, 1.59 mmmol). The reaction was monitored by ^{1}H NMR from signals of the methyl group of the acetamido moiety: CH_3CONH δ(**5**) = 1.80 and δ(**6**) = 1.82. β-D-**5** (*de* 74%). M.p. 145 °C; elemental analysis calcd (%) for $C_{53}H_{55}NO_9$ (850.0): C 74.89, H 6.52, N 1.65; found: C 74.98, H 6.45, N 1.34.

Treatment with diazomethane in CH_2Cl_2/Et_2O converted derivative **5** (*de* 74%) into the corresponding methyl ester for spectroscopic characterisation: ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.35-7.1$ (m, 26H; 5 C_6H_5, 1 × benzene ring A), 6.51 (m, 2H; benzene ring A), 5.71 (d, $J = 8.5$ Hz, 1H; NH), 4.97 (m, 2H; PhCH_2O-C_3 of benzene ring A), 4.85–4.72 (m, 5H, 4 CHHPh, CHNH), 4.62 (d, $J = 11$ Hz, 1H; CHHPh), 4.53 (d, $J = 11$ Hz, 1H; CHHPh), 4.43 (d, $J = 11$ Hz, 1H; CHHPh), 4.36 (d, $J = 11$ Hz, 1H; CHHPh), 3.66–3.22 (m, 11H; 7 × sugar moiety, $COOCH_3$, CHH-$C_{anomeric}$), 2.96 (m, 2H; CH_2CHNH), 2.59 (m, 1H; CHH-$C_{anomeric}$), 1.80 (s, 3H, NHCOCH_3). β-L-**6** (*de* > 99%). M.p. 160 °C; elemental analysis calcd (%) for $C_{53}H_{55}NO_9$ (850.0): C 74.89, H 6.52, N 1.65; found: C 75.01, H 6.48, N 1.79.

Treatment with diazomethane in CH_2Cl_2/Et_2O converted derivative **6** (*de* 99%) into the corresponding methyl ester for optical and spectroscopic characterisation: $[\alpha]_D = -4$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.35-7.1$ (m, 26H; 5 C_6H_5, 1H benzene ring A), 6.51 (m, 2H; benzene ring A), 5.74 (d, $J = 8.5$ Hz, 1H; NH), 4.97 (m, 2H; PhCH_2O-C_3 of benzene ring A), 4.85–4.72 (m, 5H; 4 CHHPh, CHNH), 4.62 (d, $J = 11$ Hz, 1H; CHHPh), 4.53 (d, $J = 11$ Hz, 1H; CHHPh), 4.43 (d, $J = 11$ Hz, 1H; CHHPh), 4.36 (d, $J = 11$ Hz, 1H; CHHPh), 3.68–3.21 (m, 11H; 7 × sugar moiety, $COOCH_3$, CHH-$C_{anomeric}$), 2.99 (m, 2H; CH_2-CH-NH), 2.96 (m, 1H; CHH-$C_{anomeric}$), 1.82 (s, 3H; NHCOCH_3).

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