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CARBOHYDRATE RESEARCH

Stereoselective synthesis of *O*-serinyl/threoninyl-2-acetamido-2-deoxy-α- or β-glycosides

Lajos Szabó, Jan Ramza, Courtney Langdon, Robin Polt *

Department of Chemistry, University of Arizona, Tucson, AZ 85721, USA

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Abstract

General glycosidation methodology has been developed which can selectively provide 2-acetamido-2-deoxy-α- or β-glycosides of β-hydroxy-α-amino acid derivatives [glucopyranoside- (**8**, **43**), galactopyranoside- (**9**, **13**), mannopyranoside- (**10**), lactoside analogs (**11**, **38**) and 3-*O*-β-galactopyranosyl-mannopyranoside (**12**)] stereoselectively in excellent yield from the highly nucleophilic α-imino esters (Schiff bases) of L-serine and L-threonine. Various glycosides were converted via their amino and acetamido derivatives to Fmoc-protected serinyl- or threoninyl-glycosides (**24–28**, **37**, **41**, **46**) which are all suitable building blocks for the solid-phase synthesis of *O*-glycopeptides. Complete ^{1}H- and ^{13}C-NMR data are provided for all compounds.

Keywords: 2-Acetamido-2-deoxy-glycosides; Stereoselective synthesis

1. Introduction

In recent years there has been increasing interest in glycoproteins, due to the central role of their carbohydrate moieties in different types of cellular recognition processes [1], in intercellular and intracellular transport of the gene products [2], in the alteration of peptide backbone conformation [3], and in the control of membrane permeability and molecular recognition [4]. Additional roles are involved in numerous disease states as the modification of the τ protein in Alzheimer's disease [5] and the antigenic T- and T_N-epitopes associated with cancer [6]. The significance of *O*-glycopeptides might be further supported by the fact that L-serinyl-β-D-glucoside enkephalin analogs are transported across the blood–brain barrier [7].

* Corresponding author.

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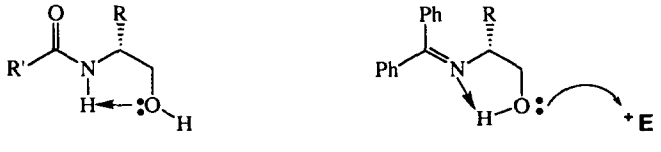
Despite the large number of naturally occurring glycoproteins, the types of covalent bonds between the protein and the saccharide moiety show limited variation. One of the most common is the *O*-linked 2-acetamido-2-deoxy-glycoside [8]. The synthesis of the *O*-glycoproteins is complicated by both acid lability of the glycosidic bond, and base sensitivity of the *O*-serinyl and *O*-threoninyl glycosides [9]. Additional complications arise due to the poor reactivity in Koenigs–Knorr reactions of the typical *N*-acylated (Boc, Cbz or Fmoc-protected) serine or threonine derivatives. Because of this poor reactivity, harsh reaction conditions are required to effect bond formation, and the yields suffer, as well as the anomer selectivity [9,10].

The poor reactivity of acyl-protected (cf. Boc, Z, etc.) serines and threonines or their analogs (cf. ceramides) is probably due to the unfavorable H-bonding pattern which arises from amide-type protecting groups. Replacement of the H-bond donor (amide) with an H-bond acceptor (imine), inverts the H-bonding pattern, and increases the nucleophilicity of the O-lone pair (Scheme 1). We have confirmed this H-bonding hypothesis by the synthesis of cerebrosides [11], *O*-serinyl and threoninyl α-or β-glycopyranosides, glycoconjugates and glycopeptides [12], as well as the related 2-deoxy-glucopyranosides [13]. We now report general synthetic methodology for Fmoc-protected *O*-serinyl/threoninyl 2-acetamido-2-deoxy-α- or β-glycopyranosides which are desirable building blocks for solid-phase *O*-linked glycopeptide synthesis [14].

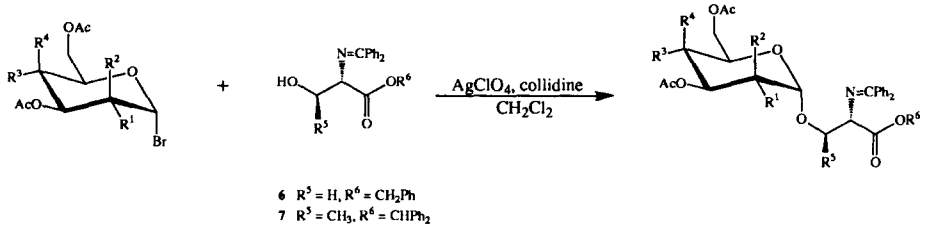
2. Results and discussion

The crucial step in the 9-fluorenylmethoxycarbonyl (Fmoc)-protected serinate- or threoninate-2-acetamido-2-deoxy-glycoside derivatives is glycosylation. For successful reaction, the highly nucleophilic α-imino esters (O'Donnell's Schiff bases [15]), benzyl *N*-(diphenylmethylene)-L-serinate (**6**) [12], and diphenylmethyl *N*-(diphenylmethylene)-L-threoninate (**7**) [12] were chosen as the glycosyl acceptors (Scheme 2).



unfavorable H-bond favorable H-bond

Scheme 1.

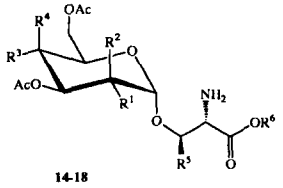


6 $R^5 = H$, $R^6 = CH_2Ph$
7 $R^5 = CH_3$, $R^6 = CHPh_2$

1 $R^1 = N_3$, $R^2 = R^4 = H$, $R^3 = OAc$
2 $R^1 = N_3$, $R^2 = R^3 = H$, $R^4 = OAc$
3 $R^1 = R^4 = H$, $R^2 = N_3$, $R^3 = OAc$
4 $R^1 = N_3$, $R^2 = R^4 = H$, $R^3 = Galp(OAc)_4$-β-O
5 $R^1 = R^4 = H$, $R^2 = N_3$, $R^3 = Galp(OAc)_4$-β-O

8 $R^1 = N_3$, $R^2 = R^4 = R^5 = H$, $R^3 = OAc$, $R^6 = CH_2Ph$
9 $R^1 = N_3$, $R^2 = R^3 = R^5 = H$, $R^4 = OAc$, $R^6 = CH_2Ph$
10 $R^1 = R^4 = R^5 = H$, $R^2 = N_3$, $R^3 = OAc$, $R^6 = CH_2Ph$
11 $R^1 = N_3$, $R^2 = R^4 = R^5 = H$, $R^3 = Galp(OAc)_4$-β-O, $R^6 = CH_2Ph$
12 $R^1 = R^4 = R^5 = H$, $R^2 = N_3$, $R^3 = Galp(OAc)_4$-β-O, $R^6 = CH_2Ph$
13 $R^1 = N_3$, $R^2 = R^3 = H$, $R^4 = OAc$, $R^5 = CH_3$, $R^6 = CHPh_2$

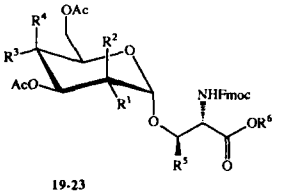
CF_3COOH, aq. THF



14-18

14 $R^1 = N_3$, $R^2 = R^4 = R^5 = H$, $R^3 = OAc$, $R^6 = CH_2Ph$
15 $R^1 = R^4 = R^5 = H$, $R^2 = N_3$, $R^3 = OAc$, $R^6 = CH_2Ph$
16 $R^1 = N_3$, $R^2 = R^4 = R^5 = H$, $R^3 = Galp(OAc)_4$-β-O, $R^6 = CH_2Ph$
17 $R^1 = R^4 = R^5 = H$, $R^2 = N_3$, $R^3 = Galp(OAc)_4$-β-O, $R^6 = CH_2Ph$
18 $R^1 = N_3$, $R^2 = R^3 = H$, $R^4 = OAc$, $R^5 = CH_3$, $R^6 = CHPh_2$

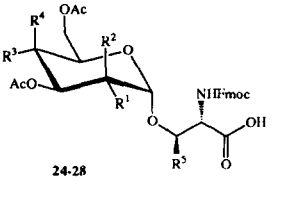
Fmoc-Cl or Fmoc-OSu, iPr_2EtN, CH_2Cl_2



19-23

19 $R^1 = N_3$, $R^2 = R^4 = R^5 = H$, $R^3 = OAc$, $R^6 = CH_2Ph$
20 $R^1 = R^4 = R^5 = H$, $R^2 = N_3$, $R^3 = OAc$, $R^6 = CH_2Ph$
21 $R^1 = N_3$, $R^2 = R^4 = R^5 = H$, $R^3 = Galp(OAc)_4$-β-O, $R^6 = CH_2Ph$
22 $R^1 = R^4 = R^5 = H$, $R^2 = N_3$, $R^3 = Galp(OAc)_4$-β-O, $R^6 = CH_2Ph$
23 $R^1 = N_3$, $R^2 = R^3 = H$, $R^4 = OAc$, $R^5 = CH_3$, $R^6 = CHPh_2$

1) H_2, Pd/C, CH_3OH, H_2O
2) Ac_2O, Et_3N, Dioxane



24-28

24 $R^1 = NHAc$, $R^2 = R^4 = R^5 = H$, $R^3 = OAc$
25 $R^1 = R^4 = R^5 = H$, $R^2 = NHAc$, $R^3 = OAc$
26 $R^1 = NHAc$, $R^2 = R^4 = R^5 = H$, $R^3 = Galp(OAc)_4$-β-O
27 $R^1 = R^4 = R^5 = H$, $R^2 = NHAc$, $R^3 = Galp(OAc)_4$-β-O
28 $R^1 = NHAc$, $R^2 = R^3 = H$, $R^4 = OAc$, $R^5 = CH_3$

Scheme 2.

Table 1
^{1}H-NMR data for glycosides [a]

Chemical shifts (ppm) Number	8 [b]	9	10 [b]	13	14	15	18	19	20	23	30	43	46
H-1	4.58	5.03	4.56	5.27	4.97	4.51	4.81	4.83	4.32	4.66	4.81	4.66	4.51
H-2	2.77	3.63	3.50	3.65	3.30	3.57	3.49	3.25	3.43	3.39	4.50	3.53	3.83
H-3	5.63	5.24	5.56	5.26	5.43	5.47	5.22	5.40	5.44	5.21	5.04	5.06	5.08
H-4	5.12	5.32	5.71	5.38	5.04	5.61	5.38	4.98	5.58	5.38	5.26	4.94	5.01
H-5	3.81	4.00	3.88	4.22	4.04	3.98	4.18	3.94	3.95	4.26	4.00	3.54	3.68
H-6	4.26	4.01	4.30	4.05	4.26	4.27	4.03	4.18	4.26	4.43	4.00	4.13	4.19
H-6′	3.94	3.99	4.10	4.05	4.08	4.19	4.03	4.07	4.26	4.37	4.00	3.98	3.99
α-H	4.68	4.44	4.69	4.25	3.75	3.32	3.50	4.61	4.55	4.53	4.36	4.67	5.16
β-H	4.22	4.21	4.35	4.46	4.01	3.68	4.23	4.17	3.66	4.48	4.17	4.28	4.01
β′-H	3.96	3.94	3.91		3.87	3.46		4.01	3.66		3.81	4.28	3.69
CH_2Ph	4.98	5.16	5.07		5.20	5.03		5.24	4.93		5.16	5.06	
$CHPh_2$				6.88			6.96			6.97			
CH_3				1.17			1.34			1.34			
NH								5.91	6.06	5.74	5.59	5.21	5.94
$Fmoc_1$								4.23	4.10	4.12			4.20
$Fmoc_{2\text{-}3}$								4.41	4.42	4.05			4.68
										4.03			4.63
CH_2												4.02	

[a] NMR data in $CDCl_3$ solution unless otherwise indicated.
[b] Solution was C_6D_6.

Table 2
^{1}H-NMR data for glycosides

Coupling constants (Hz) $J_{H,H}$	8	9	10	13	14	15	18	19	20	23	30	43	46
1,2	3.5	3.5	1.4	3.6	3.6	1.4	3.7	3.4	0.9	3.7	3.6	8.5	8.4
2,3	10.6	9.0	3.8	11.1	10.6	3.8	11.1	10.6	3.7	11.1	11.2	10.0	9.3
3,4	9.5	3.3	9.9	3.2	9.3	9.9	3.2	9.8	9.9	2.4	3.2	10.1	9.2
4,5	10.3	1.2	9.9	nd	10.2	9.9	0.9	10.3	9.8	nd	nd	9.8	9.4
5,6	4.3	5.9	4.5	6.8	4.6	4.4	6.1	5.2	3.5	7.6	nd	4.5	4.2
5,6′	2.2	nd	2.1	6.8	2.2	2.2	6.1	1.6	3.5	7.3	nd	2.2	2.0
6,6′	−12.6	nd	−12.3	nd	−12.5	−12.3	nd	−12.3	nd	−10.5	nd	−12.3	−12.2
α,β	4.3	4.5	4.6	7.6	3.7	4.5	3.0	2.5	4.3	1.9	5.2	4.6	5.9
α,β′	8.2	7.7	8.0		5.0	4.9		2.5	3.9		7.0	5.5	5.9
β,β′	9.7	9.8	9.5		9.9	9.6		10.9	11.5		9.8	10.4	11.0
β,CH_3				6.3			6.5			6.5			
NH-α								8.1	8.2	9.5			5.9
NH-2											9.8	8.6	9.1
$Fmoc_{1\text{-}2}$								7.1	6.5	5.9			5.5
$Fmoc_{1\text{-}2'}$								7.1	6.5	7.1			5.4
$Fmoc_{2\text{-}2'}$								nd	nd	11.2			nd

Table 3
^{1}H-NMR data for glycosides

Chemical shifts (ppm) Number	11	12	16	17	21	22	38	39	40
H-1	4.98	4.80	4.92	4.68	4.79	4.59	4.43	4.37	4.31
H-2	3.21	3.89	3.12	3.91	3.08	3.66	3.33	3.37	3.35
H-3	5.38	5.32	5.40	5.31	5.40	5.31	4.95	4.96	4.96
H-4	3.68	3.94	3.71	3.92	3.69	3.88	3.67	3.69	3.67
H-5	3.83	3.75	3.95	3.85	3.90	3.79	3.45	3.51	3.46
H-6a	4.38	4.37	4.45	4.40	4.48	4.41	4.34	4.46	4.45
H-6b	4.11	4.10	4.13	4.11	4.17	4.07	4.04	4.08	4.07
H-1′	4.45	4.51	4.47	4.52	4.50	4.55	4.41	4.45	4.44
H-2′	5.12	5.14	5.10	5.12	5.13	5.13	5.07	5.08	5.09
H-3′	4.95	4.97	4.95	4.98	4.97	4.99	4.93	4.95	4.95
H-4′	5.35	5.37	5.34	5.36	5.36	5.37	5.34	5.35	5.35
H-5′	3.84	3.90	3.87	3.90	3.86	3.92	3.84	3.87	3.87
H-6′a	4.17	4.18	4.19	4.18	4.21	4.19	4.15	4.16	4.17
H-6′b	4.08	4.09	4.08	4.09	4.06	4.10	4.07	4.08	4.08
α-H	4.44	4.40	3.83	3.72	4.62	4.61	4.45	3.72	4.62
β-H	4.23	4.18	4.00	3.75	4.08	4.37	4.17	4.17	4.36
β′-H	3.94	3.87	3.73	3.71	3.98	4.01	4.05	3.86	3.89
CH_2Ph	5.16	5.15	5.18	5.19		5.11	5.15	5.18	5.22
$Fmoc_1$					4.25	4.26			4.23
$Fmoc_2$					4.25	4.26			4.38

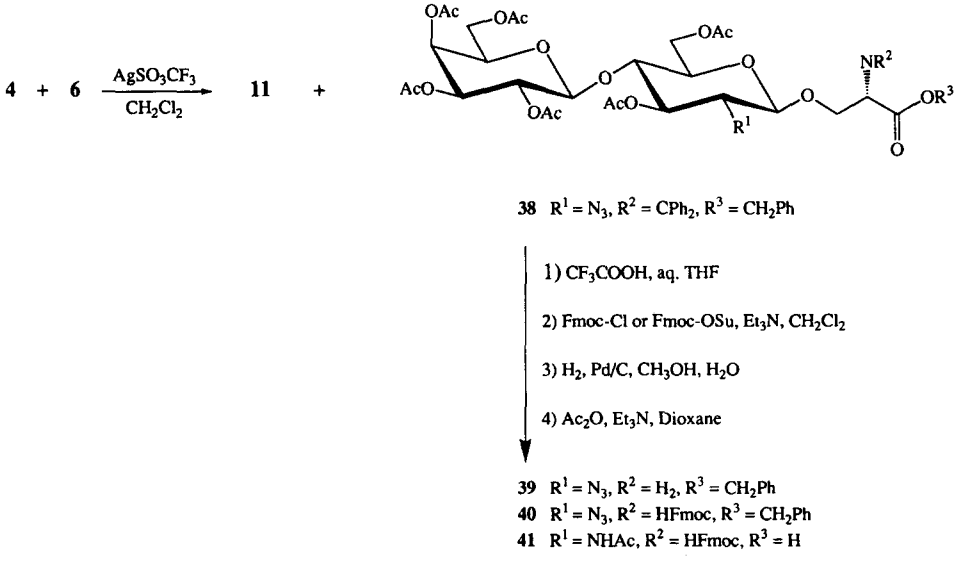
Table 4
^{1}H-NMR data for glycosides

Coupling constants (Hz) $J_{H,H}$	11	12	16	17	21	22	38	39	40
1,2	3.6	1.5	3.5	1.6	3.6	2.3	8.0	8.0	8.0
2,3	10.7	3.9	10.6	3.8	10.7	3.7	10.2	10.3	9.5
3,4	9.0	9.1	9.4	8.6	9.8	8.6	10.0	9.9	9.6
4,5	9.6	9.5	9.4	9.1	9.5	9.3	9.7	9.6	9.6
5,6a	2.1	1.8	2.4	1.6	2.2	2.3	2.8	1.8	1.6
5,6b	4.8	4.9	4.7	5.4	4.2	4.7	4.9	5.0	4.7
6a,6b	−12.1	−11.9	−12.1	−12.0	−11.8	−11.9	−12.0	−12.1	−12.4
1′,2′	7.9	7.9	7.9	8.0	7.8	8.0	7.9	7.7	7.9
2′,3′	10.4	9.3	10.2	10.4	10.4	10.5	10.4	10.3	10.4
3′,4′	3.5	3.2	3.5	3.4	3.4	3.4	3.5	3.3	3.5
4′,5′	0.9	0.7	nd	nd	nd	nd	1.3	nd	nd
5′,6′a	6.2	6.6	6.0	6.6	6.0	6.6	6.5	6.2	6.3
5′,6′b	7.6	7.0	7.8	7.7	5.9	6.8	7.3	6.4	7.2
6′a,6′b	11.2	11.1	11.0	11.2	10.9	11.1	11.1	11.1	11.2
α,β	4.4	4.2	3.5	3.0		2.9	7.0	4.6	2.9
α,β′	8.1	8.5	4.9	5.1		3.0	6.2	4.5	3.0
β,β′	9.8	9.9	9.8	12.3		10.6	8.1	9.9	8.3
β,CH_3						8.3			8.3

For the synthesis of α-*O*-glycosides, per-*O*-acetyl 2-azido-2-deoxy-glycopyranosyl halides were selected as glycosyl donors [16], including non-participating groups at C-2. Thus, the Schiff bases **6** and **7** were treated with various acyl-protected 2-azido-glycosylbromides (**1** [17], **2** [18], **3** [17], **4** [19], **5** [19]) in the presence of silver perchlorate [20] and 2,4,6-trimethylpyridine using dichloromethane as a solvent at room temperature to provide the desired α-glycosides **8–13** in excellent yield (73–87%). The glycosides were purified on SiO_2 by flash chromatography. The corresponding β-anomer could not be detected by either thin-layer chromatography or 250 MHz ^{1}H-NMR. The α-*O*-threoninyl-glycoside **7** was less reactive due to the secondary OH group. After azidonitrationof the glucal [18], the two products, the *gluco* and the *manno* analogs, were not separated, but directly converted [17] to the corresponding bromides **1** and **3**, and reacted with the serine Schiff base **6**. The products, **8** and **10,** were easily separated by column chromatography to give the pure glycosides. All of the ^{1}H- and ^{13}C-NMR data (Tables 1–6) were consistent with the expected structures, and most of the chemical shift assignments were made by COSY or HETCOR methods. The anomeric ratios and assignments were made via ^{1}H–^{1}H spin-coupling constants ($^3J_{H1,H2}$ 3–4 Hz for α-anomers). ^{13}C-NMR chemical shifts, and proton–carbon one-bond coupling constants (for α-anomers, $^1J_{C1,H1} \sim 170$ Hz [21]) were also recorded. The last data confirmed the *manno* configurations as well.

The glycosylation of serinyl Schiff base **6** with hexa-*O*-acetyl-2-azido-2-deoxy-lactosyl bromide **4** [19] (Scheme 3) using Hanessian's modification of the Koenigs–Knorr reaction [22] provided a mixture (6:1) of the α-lactoside **11**, and the 2-azido-2-deoxy-β-lactoside **38** which were separable by flash chromatography on SiO_2.

In our first approach to the synthesis of Fmoc protected glycosides, the azido group was converted to the acetamido derivative by the Staudinger reaction with tri-



Scheme 3.

Table 5
^{13}C-NMR shifts in ppm for glycosides [a]

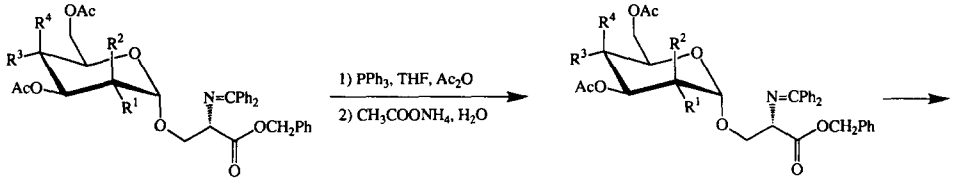
Number	8	9	10 [b]	11	12	13	14	15	16	17	18	19	20	21
C-1	97.46	98.10	97.80	97.58	97.99	98.45	98.26	98.05	98.25	97.70	99.05	99.02	98.67	99.00
C-2	61.10	57.57	61.30	61.11	61.27	57.80	60.55	60.79	60.75	60.69	57.74	60.76	60.94	60.80
C-3	70.36	67.57	70.97	69.81	70.96	67.13	69.88	70.49	69.56	70.39	67.44	69.97	70.59	69.52
C-4	67.79	66.77	65.65	76.09	73.76	66.26	67.58	65.47	75.91	73.44	66.71	67.22	65.67	76.09
C-5	68.22	68.10	68.80	68.42	69.21	68.11	68.05	68.38	68.42	68.90	68.41	68.14	68.87	68.72
C-6	61.25	61.63	61.45	61.61	62.01	61.29	61.43	61.70	61.54	61.66	61.71	61.65	61.88	61.74
C-1′				100.77	101.17				100.67	100.65				100.72
C-2′				68.87	69.01				68.82	68.64				68.88
C-3′				70.88	70.96				70.67	70.39				70.70
C-4′				66.42	66.71				66.35	66.47				66.39
C-5′				70.38	70.56				70.32	70.13				70.39
C-6′				60.56	61.01				60.53	60.81				60.54
C=N	172.68	172.82	172.12	173.15	173.04	171.56								
α-C	65.20	65.22	65.27	64.94	65.01	70.99	54.38	54.09	54.36	53.96	59.91	54.48	54.18	54.34
β-C	68.85	69.07	68.25	68.70	68.55	76.75 [c]	70.71	69.99	70.55	69.84	78.14 [c]	69.85	69.24	69.63
CH_2Ph	66.25	66.87	66.35	66.67	66.76		66.94	66.73	66.87	66.38		68.29	66.91	67.08
$CHPh_2$						76.70 [c]					77.73 [c]			
CH_3						17.89					18.66			
CH_2Fmoc												68.06	67.20	67.52
CHFmoc												47.00	46.94	46.81
J_{C1-H1}		171.5	173.9	172.8		174.1				172.0				
$J_{C1'-H1'}$				160.1						158.3				

[a] MNR data in $CDCl_3$ solution unless otherwise indicated.
[b] Solution was C_6D_6.
[c] Assignments in the same column may be reversed.

Table 6

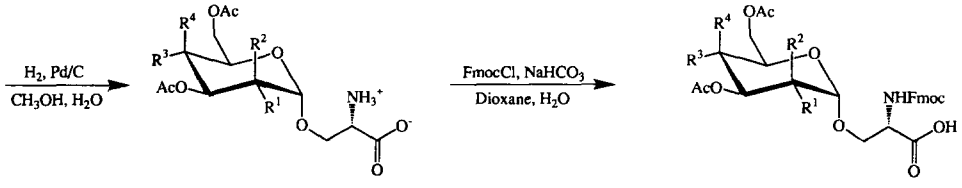
Number	22	23	24	25	26 [a]	27	28 [a]	30	34 [b]	37	38	39	40	41
C-1	98.67	98.38	98.2	98.7	97.72	99.58	98.78	98.30	97.99	98.55	100.70	100.52	100.62	100.49
C-2	60.73	57.53	51.9	53.1	50.88	49.97	46.76	47.58	47.20	47.03	63.86	63.66	63.64	53.59
C-3	70.70	67.23	72.5	68.5	69.61	70.77	67.23	67.04	66.72	65.08	71.76	71.40	71.39	71.49
C-4	74.05	66.78	67.1	62.4	76.43	74.55	66.44	67.37	67.61	67.19	75.67	75.55	75.61	76.46
C-5	69.41	68.21	69.9	67.2	68.22	69.10	67.67	68.42	68.05	68.08	72.41	72.35	72.47	72.38
C-6	61.96	61.56	61.7	60.9	62.00	62.52	61.69	61.90	62.11	61.88	61.61	61.53	61.60	62.09
C-1′	101.07				100.08	100.84					101.43	101.46	101.53	101.51
C-2′	68.89				68.99	68.91					68.83	68.73	68.83	68.80
C-3′	70.70				70.73	70.37					70.76	70.61	70.69	70.62
C-4′	66.64				67.63	66.60					66.44	66.38	66.46	66.44
C-5′	70.50				70.37	69.95					70.44	70.35	70.46	70.28
C-6′	60.98				60.83	60.80					60.70	60.70	60.80	60.67
C=N											172.26			
α-C	54.14	58.66	54.3	54.1	54.31	54.06	58.45	65.13	52.84	54.33	65.15	54.33	53.92	53.59
β-C	69.26	76.09 [c]	70.5	66.2	65.76	69.14	74.92	69.16	65.42	69.33	70.42	71.66	69.50	68.31
CH_2Ph	67.12							66.98			66.63	66.57	67.00	
$CHPh_2$		78.39 [c]												
CH_3		18.47					18.55							
CH_2Fmoc	67.26	67.44	67.9	60.0	67.05	67.40	68.46			67.19			67.14	65.84
CHFmoc	46.79	46.95	47.1	46.9	46.61	46.95	46.55			47.99			46.76	46.68
J_{C1-H1}									172.0		160.5		159.3	
$J_{C1'-H1'}$											161.5		158.3	

[a] Solution was Me_2SO.
[b] Solution was D_2O.
[c] Assignments in the same column may be reversed.



8 $R^1 = N_3$, $R^2 = R^4 = H$, $R^3 = OAc$
9 $R^1 = N_3$, $R^2 = R^3 = H$, $R^4 = OAc$
11 $R^1 = N_3$, $R^2 = R^4 = H$, $R^3 = Galp(OAc)_4$-β-O
12 $R^1 = R^4 = H$, $R^2 = N_3$, $R^3 = Galp(OAc)_4$-β-O

29 $R^1 = NHAc$, $R^2 = R^4 = H$, $R^3 = OAc$
30 $R^1 = NHAc$, $R^2 = R^3 = H$, $R^4 = OAc$
31 $R^1 = NHAc$, $R^2 = R^4 = H$, $R^3 = Galp(OAc)_4$-β-O
32 $R^1 = R^4 = H$, $R^2 = NHAc$, $R^3 = Galp(OAc)_4$-β-O



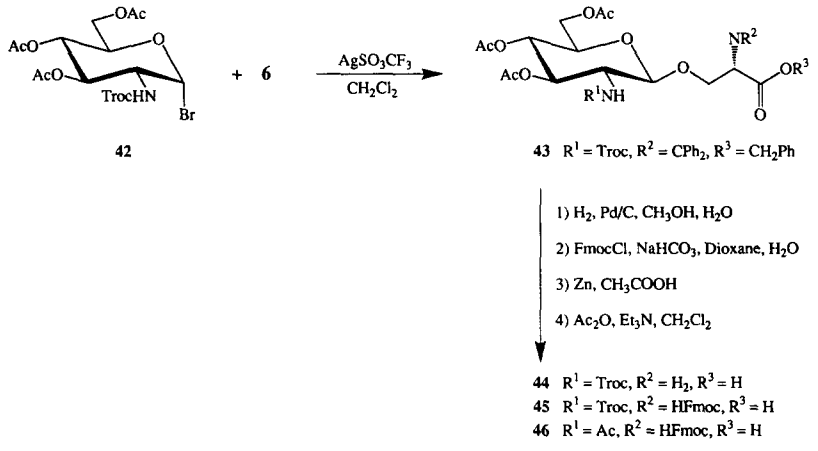
33 $R^1 = NHAc$, $R^2 = R^4 = H$, $R^3 = OAc$
34 $R^1 = NHAc$, $R^2 = R^3 = H$, $R^4 = OAc$
35 $R^1 = NHAc$, $R^2 = R^4 = H$, $R^3 = Galp(OAc)_4$-β-O
36 $R^1 = R^4 = H$, $R^2 = NHAc$, $R^3 = Galp(OAc)_4$-β-O

24 $R^1 = NHAc$, $R^2 = R^4 = H$, $R^3 = OAc$
37 $R^1 = NHAc$, $R^2 = R^3 = H$, $R^4 = OAc$
26 $R^1 = NHAc$, $R^2 = R^4 = H$, $R^3 = Galp(OAc)_4$-β-O
27 $R^1 = R^4 = H$, $R^2 = NHAc$, $R^3 = Galp(OAc)_4$-β-O

Scheme 4.

phenylphosphine and acetic anhydride [23]. Since the Schiff base moiety is acid sensitive, the triphenylphosphineimine was cleaved in 1 M ammonium acetate buffer (Scheme 4) to give the 2-acetamido-2-deoxy analogues **29–32**. In the next step, the Schiff base and the benzyl ester were reduced with Pd/C under an H_2 atmosphere, followed by Fmoc acylation in presence of sodium bicarbonate to provide the desired Fmoc acetamido-glycosides **24**, **26**, **27** and **37** [27]. The intermediate glycoside esters were all purified and fully characterized, except for the azido glucoside, which was converted in a one pot reaction to **24**. This route was marginally satisfactory, but required long reaction times (several days), and the separation of the phosphine oxide was difficult. Thus, we developed a second, more efficient route to the Fmoc amino acid glycosides.

Following the improved route, the Schiff base was cleaved within 5–10 min using 10% trifluoroacetic acid to provide the amino glycosides **14–18** and **39** (Scheme 2). Tetrahydrofuran proved to be a better solvent than dichloromethane for this cleavage reaction. The free amino group was converted to the Fmoc-derivatives **19–23** and **40** in organic solvent in the presence of diisopropylethylamine or triethylamine within 15–30 min. The fluorenylmethoxycarbonyl group was very stable (despite expected cleavage [24]) during the hydrogenation of azido moiety of **19–23** and **40**. The amines were converted to the acetamido derivatives **24–28** [27] and **41** by acetic anhydride in the presence of an organic base. The above four reaction steps were nearly quantitative, and



Scheme 5.

required only 4–5 h. The last two reactions were followed by TLC strictly to avoid the possible migration of the Fmoc group observed after longer reaction times. In one experiment the protected threoninyl-galactopyranoside **13** was transformed to the desired Fmoc-analog **28** without any intermediate chromatographic purification. The target compound **28** was isolated on a short column to give a 92% yield for the four steps. Thus, this method appears more practical than the Staudinger route.

For the synthesis of 1,2 *trans-O*-glycosides (β-anomer in the *gluco* series) the (2,2,2-trichloroethoxy)carbonyl (Troc) participating protecting group was used on the glycosyl donor **42** [25]. Glycosylation with the nucleophilic Schiff base [17] **6** using Hanessian's reaction [22] gave the β-serinyl-D-glucoside derivatives **43** an excellent yield as we have reported in one similar reaction [12] (Scheme 5). The conversion of **43** to (*N*-Fmoc-L-serin-3-yl)-3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside **46** has also been demonstrated. The Schiff base and the benzyl groups were cleaved from **43** by hydrogen in the presence of Pd/C, followed by re-protection of the amino group with Fmoc-Cl to provide **45**. The Troc protecting group was transformed [26] to the unprotected amine with zinc/metal in acetic acid after subsequent treatment with acetic anhydride, the desired **46** was obtained in 62% overall yield.

3. Experimental

General methods.—All air and moisture sensitive reactions were performed under an argon atmosphere in flame-dried reaction flasks. THF was dried and de-oxygenated over $Ph_2C{=}O/Na°–K°$. CH_2Cl_2 and CH_3CN were dried over P_2O_5 and all solvents were freshly distilled under an argon atmosphere prior to use. For flash chromatography, 230–400 mesh silica gel 60 (E. Merck No. 9385) was employed. All compounds described were > 95% pure by ^{1}H- and ^{13}C-NMR, and purity was confirmed by elemental analysis in many cases. The ^{1}H- and ^{13}C-NMR spectra were obtained on a

Bruker WM- or AM-250 MHz and a Gemini 200 MHz spectrometer. COSY and HETCOR spectra were obtained on a Bruker WM-500 spectrometer at 500 MHz or VARIAN UNITY 300 spectrometer at 300 MHz. Chemical shifts are reported in δ using Me_4Si as the standard reference in ^1H spectra and $CDCl_3$ in ^{13}C spectra. Infrared spectra were obtained on a Perkin–Elmer 1600 Series FT-IR. All melting points were measured on a Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured on a Randolph Research AutoPol III polarimeter using the Na–D line. Elemental analyses were performed by Desert Analytics, Tucson, AZ 85719. Nominal and exact mass spectra were obtained on a JEOL JMS-01SG-2 mass spectrometer.

Reaction of acetobromoglycopyranose (**1–5**) *and serine* (**6**) *or threonine Schiff base ester* (**7**) *using* $AgClO_4$ *(Method A —selective for α-glycosides).*—A mixture of bromide (**1** [17], **2** [18], **3** [17], **4** [19], or **5** [19]) (1.4 equiv), aglycone (**5** [12] or **6** [12]) (1 mmol), and 2,4,6-collidine (1.7 equiv) in dichloromethane (5 mL) was dropped into a rigorously stirred suspension of $AgClO_4$ (1.7 equiv) in dichloromethane (10 mL) over a period of 40 min at room temperature. After the addition was complete the reaction was stirred for an additional 15 min, then quenched with Et_3N (2 equiv), diluted with dichloromethane (100 mL), and filtered through Celite. The filtrate was washed with 5% $Na_2S_2O_3$ solution (3 × 10 mL), saturated $NaHCO_3$ solution (3 × 10 mL), and water (3 × 10 mL). The colorless solution was dried over Na_2SO_4, evaporated under vacuum and separated by flash chromatography to provide glycosides **8–13**.

N-*Diphenylmethylene*-O-*(3,4,6-tri*-O-*acetyl-2-azido-2-deoxy-α*-D-*glucopyranosyl* (**8**) *and mannopyranosyl*(**10**))-L-*serine benzyl ester.*—An unseparated mixture of **1** [17] and **3** [17] from the azidonitration [18] was reacted with **6** [12] as above to provide a mixture of *gluco* and *manno* products which could be easily separated on a SiO_2 column. The first fraction provided the *manno* product **10**. Syrup, 24%, $[\alpha]_D$ +34° (*c* 1.9, chloroform), R_f 0.50 (hexane–ethylacetate 1:1). ^1H- and ^{13}C-NMR data are provided in Tables 1–6. Anal. Calcd for $C_{35}H_{36}O_{10}N_4$: C, 62.48; H, 5.40; N, 8.33. Found: C, 62.37; H, 5.48; N, 8.28.

The second fraction provided the *gluco* product **8**. Syrup, 49%, $[\alpha]_D$ +80° (*c* 0.4, chloroform), R_f 0.45 (hexane–ethylacetate 1:1). ^1H- and ^{13}C-NMR data are provided in Tables 1–6. Anal. Calcd for $C_{35}H_{36}O_{10}N_4$: C, 62.48; H, 5.40; N, 8.33. Found: C, 62.51; H, 5.52; N, 8.20.

N-*Diphenylmethylene*-O-*(3,4,6-tri*-O-*acetyl-2-azido-2-deoxy-α*-D-*galactopyranosyl)*-L-*serine benzyl ester* (**9**).—Syrup, 82%, $[\alpha]_D$ +38.2° (*c* 0.45, chloroform R_f 0.44 (dichloromethane–ethylacetate, 93:7). ^1H- and ^{13}C-NMR data are provided in Tables 1–6. Anal. Calcd for $C_{35}H_{36}O_{10}N_4$: C, 62.48; H, 5.40; N, 8.33. Found: C, 62.44; H, 5.47; N, 8.42.

N-*Diphenylmethylene*-O-*[*O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-3,6-di*-O-*acetyl-2-azido-2-deoxy-α*-D-*glucopyranosyl]*-L-*serine benzyl ester* (**11**) *(Method A).*—Foam, 81%, $[\alpha]_D$ +18.3° (*c* 1.8, chloroform), R_f 0.35 (hexane–ethylacetate 55:45). ^1H- and ^{13}C-NMR data are provided in Tables 1–6. Anal. Calcd for $C_{47}H_{52}O_{18}N_4$: C, 58.73; H, 5.46; N, 5.83. Found: C, 58.69; H, 5.55; N, 5.68.

N-Diphenylmethylene-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl]-L-serine benzyl ester (**12**).—Foam, 87%, $[\alpha]_D$ +12.7° (*c* 0.7, chloroform), R_f 0.47 (toluene–ethylacetate 6:4). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. Anal. Calcd for $C_{47}H_{52}O_{18}N_4$: C, 58.73; H, 5.46; N, 5.83. Found: C, 58.80; H, 5.51; N, 5.72.

N-Diphenylmethylene-O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-threonine diphenylmethyl ester (**13**).—Syrup, 76%, $[\alpha]_D$ +4.6° (*c* 1.5, chloroform), R_f 0.28 (hexane–ethylacetate, 75:25). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. Anal. Calcd for $C_{42}H_{42}O_{10}N_4$: C, 66.13; H, 5.55; N, 7.34. Found: C, 65.94; H, 5.72; N, 7.20.

N-Diphenylmethylene-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-azido-2-deoxy-α-(**11**) *and β-*(**38**)*D-glucopyranosyl]-L-serine benzyl ester (Method B —preparation of authentic β-glycoside).*—Benzyl *N*-(diphenylmethylene)-L-serinate (**6**) [12] (1.965 g), bromide (**4**) [19] (4.5 g), powdered, oven-dried 4 Å molecular sieves (5 g), and dichloromethane (25 mL) were stirred at 0°C under argon for 10 min. Silver triflate (1.8 g) was added in portions over 15 min, and stirring was continued for 14 h at room temperature. The reaction was quenched with Et_3N (2 mL), diluted with dichloromethane (150 mL), filtered through Celite and the organic layer was washed with saturated $NaHCO_3$ (3 × 15 mL), H_2O (3 × 15 mL), and dried ($MgSO_4$). Rotary evaporation and flash chromatography on SiO_2 with hexanes–ethylacetate, 55:45 (R_f 0.41) provided 320 mg pure **38** as a syrup, and a cofraction consisting of a mixture of **38** and **11**. The cofraction was re-chromotagraphed, to provide an additional 310 mg, thus giving 630 mg **38** (12%) in toto, $[\alpha]_D$ −8.2° (*c* 0.2, chloroform). ^{1}H- and ^{13}C-NMR data are in Tables 1–6. Anal. Calcd for $C_{47}H_{52}O_{18}N_4$: C, 58.73; H, 5.46; N, 5.83. Found: C, 58.85; H, 5.60; N, 5.60.

The second fraction (R_f 0.35) provided 3.4 g **11** (71%) after two separations. Anal. Found: C, 58.68; H, 5.50; N, 5.76.

Removal of Schiff base from the serine / threonine-glycoside.—Glycoside **8, 10–13** or **38** (1 mmol) was dissolved in tetrahydrofuran (9 mL), and trifluoroacetic acid (1 mL) and water (0.2 mL) were added. The hydrolysis was complete within 5 min. The solution was diluted with dichloromethane (100 mL), washed by saturated $NaHCO_3$ solution (3 × 10 mL), water (3 × 10 mL), dried (Na_2SO_4), and evaporated in vacuo. The syrup was separated on a short SiO_2 column with a gradient of dichloromethane with 0.5% Et_3N to dichloromethane–acetone, 75:25 with 0.5% Et_3N to provide the amino esters **14–18** and **39**. Since the benzyl esters are unstable as the free base [12] (diketopiperazine formation), no attempt was made to obtain elemental analyses on these intermediates.

O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-L-serine benzyl ester (**14**).—Syrup, 94%, $[\alpha]_D$ +136° (*c* 0.25, chloroform). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{22}H_{28}O_{10}N_4$ 508.2, found *m/z* 509.2 (MH^+).

O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl)-L-serine benzyl ester (**15**).—Syrup, 92%, $[\alpha]_D$ +63.6° (*c* 0.27, chloroform). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{22}H_{28}O_{10}N_4$ 508.2, found *m/z* 509.2 (MH^+).

O-*[*O-*(2,3,4,6-Tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)-3,6-di*-O-*acetyl-2-azido-2-deoxy-α-*D-*glucopyranosyl]-*L-*serine benzyl ester* (**16**).—Syrup, 97%, $[\alpha]_D$ +70.5° (*c* 1.6, chloroform). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{34}H_{44}O_{18}N_4$ 796.2, found *m/z* 797.2 (MH^+).

O-*[(2,3,4,6-Tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)-3,6-di*-O-*acetyl-2-azido-2-deoxy-α-*D-*mannopyranosyl]-*L-*serine benzyl ester* (**17**).—Syrup, 96%, $[\alpha]_D$ +33.9° (*c* 2.7, chloroform). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{34}H_{44}O_{18}N_4$ 796.2, found *m/z* 797.1 (MH^+).

O-*(3,4,6-Tri*-O-*acetyl-2-azido-2-deoxy-α-*D-*galactopyranosyl)-*L-*threonine diphenylmethyl ester* (**18**).—Glassy solid, 91%, $[\alpha]_D$ +40.9° (*c* 1.1, chloroform). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{29}H_{34}O_{10}N_4$ 598.2, found *m/z* 599.2 (MH^+).

O-*[*O-*(2,3,4,6-Tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)-3,6-di*-O-*acetyl-2-azido-2-deoxy-β-*D-*glucopyranosyl]-*L-*serine benzyl ester* (**39**).—Syrup, 96%, $[\alpha]_D$ +0.3° (*c* 3.0, chloroform). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{34}H_{44}O_{18}N_4$ 796.2, found *m/z* 797.1 (MH^+).

Fmoc-protection of the serinate / threoninate glycoside esters.—Compound **14–18**, or **39** (1 mmol) was dissolved in dichloromethane (10 mL) and treated with 9-fluorenylmethylchloroformate or *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (1 mmol) in the presence of *N,N*-diisopropylethylamine (2 mmol) for 45 min at room temperature. The solution was then diluted with dichloromethane (100 mL), washed with saturated $NaHCO_3$ solution (3 × 10 mL), water (3 × 10 mL), dried (Na_2SO_4), evaporated under vacuum, and separated on a SiO_2 column to provide the Fmoc-esters **19–23** or **40**.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(3,4,6-tri*-O-*acetyl-2-azido-2-deoxy-α-*D-*glucopyranosyl)-*L-*serine benzyl ester* (**19**).—Foam, 92%, $[\alpha]_D$ +92° (*c* 2.7, chloroform), R_f 0.25 (toluene–ethylacetate 2:1). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{37}H_{38}O_{12}N_4$ 730.2, found *m/z* 731.2 (MH^+).

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(3,4,6-tri*-O-*acetyl-2-azido-2-deoxy-α-*D-*mannopyranosyl)-*L-*serine benzyl ester* (**20**).—Foam, 87%, $[\alpha]_D$ +51.3° (*c* 0.15, chloroform), R_f 0.30 (toluene–ethylacetate 2:1). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{37}H_{38}O_{12}N_4$ 730.2, found *m/z* 731.2 (MH^+).

N-*(9-Florenylmethoxycarbonyl)*-O-*[*O-*(2,3,4,6-tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)-3,6-di*-O-*acetyl-2-azido-2-deoxy-α-*D-*glucopyranosyl]-*L-*serine benzyl ester* (**21**).—Foam, 95%, $[\alpha]_D$ +62.8° (*c* 0.6, chloroform), R_f 0.30 (toluene–ethylacetate 6:4). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{49}H_{54}O_{20}N_4$ 1018.3, found *m/z* 1019.3 (MH^+).

N-*(9-Fluorenylmethoxycarbonyl)*-O-*[*O-*(2,3,4,6-tetra*-O-*acetyl-β-*D-*galactopyranosyl)-(1 → 4)-3,6-di*-O-*acetyl-2-azido-2-deoxy-α-*D-*mannopyranosyl]-*L-*serine benzyl ester* (**22**).—Syrup, 96%, $[\alpha]_D$ +31.7° (*c* 1.2, chloroform), R_f 0.50 (toluene–ethylacetate, 6:4). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{49}H_{54}O_{20}N_4$ 1018.3, found *m/z* 1019.4 (MH^+).

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(3,4,6-tri*-O-*acetyl-2-azido-2-deoxy-α-*D-*galactopyranosyl)-*L-*threonine diphenylmethyl ester* (**23**).—Syrup, 91%, $[\alpha]_D$ +53.7° (*c* 0.5, chloroform), R_f 0.31 (hexane–ethylacetate 6:4). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{44}H_{44}O_{12}N_4$ 820.2, found *m/z* 821.3 (MH^+).

N-(9-Fluorenylmethoxycarbonyl)-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-azido-2-deoxy-β-D-glucopyranosyl]-L-serine benzyl ester (**40**).—Amorphous solid, 92%, $[\alpha]_D$ +4.9° (*c* 2.9, chloroform), R_f 0.41 (toluene–ethylacetate, 6:4). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. FAB-MS: Calcd for $C_{49}H_{54}O_{20}N_4$ 1019.3, found *m/z* 1019.3 (MH^+).

Fmoc-amino acid glycosides. Azide reduction / acylation, and ester hydrogenolysis. —Compound **19–23**, or **40** (0.5 mmol) was dissolved in methanol (150 mL), and water (10 mL) and 5% palladium on activated carbon (200 mg) were added. This mixture was vigorously stirred under H_2 (1 atm) for 1–2 h (followed by TLC). The Pd/C was filtered from the suspension, and the solution was evaporated in vacuo. The residue was stirred in dioxane (20 mL) in the presence of acetic anhydride (1.3 equiv) and Et_3N (3 equiv) for about 30 min. at room temperature (followed by TLC). The mixture was concentrated in vacuo, dissolved in dichloromethane (100 mL). The pH was set to 3 with acetic acid and the solution was washed with water (3 × 10 mL), dried over $MgSO_4$, evaporated, and re-evaporated from toluene under vacuum. The residue was separated on a short SiO_2 column to provide **24–28**, and **41**. Since the Fmoc-amino acid derivatives exist as a mixture of rotamers, only characteristic ^{1}H-NMR data are presented.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-serine (**24**).—Oil, 88%, $[\alpha]_D$ +68° (*c* 2.2 chloroform), R_f 0.33 (dichloromethane–methanol 9:1). Characteristic ^{1}H-NMR data : δ 7.60–7.03 (m, 8 H, arom.), 6.35 (d, 1 H, *J* 9.8 Hz, NH), 6.24 (d, 1 H, *J* 9.6 Hz, NH), 1.98, 1.97, 1.90, 1.85 (4s, 12 H, 4 CH_3CO). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Calcd for $C_{32}H_{36}O_{13}N_2$: C, 58.53; H, 5.53; N, 4.27. Found: C, 58.66; H, 5.45; N, 4.12.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl)-L-serine (**25**).—Oil, 90%, $[\alpha]_D$ +48.9° (*c* 0.55 chloroform), R_f 0.2 (dichloromethane–methanol 9:1). Characteristic ^{1}H-NMR data : δ 7.70–7.05 (m, 8 H, arom.), 6.26 (d, 1 H, NH), 5.62 (d, 1 H, NH), 4.92 (dd, 1 H, $J_{2,3} = J_{3,4}$ 10.4 Hz, H-3), 2.08, 1.97, 1.96, 1.95 (4s, 12 H, 4 CH_3CO). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Calcd for $C_{32}H_{36}O_{13}N_2$: C, 58.53; H, 5.53; N, 4.27. Found: C, 58.40; H, 5.38; N, 4.32.

N-(9-Fluorenylmethoxycarbonyl)-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl]-L-serine (**26**).—Amorphous solid, 98%, $[\alpha]_D$ +55.5° (*c* 0.7 chloroform), R_f 0.5 (dichloromethane–methanol 85:15). Characteristic ^{1}H-NMR data (d_6-Me_2SO) : δ 7.89–7.31 (m, 8 H, arom.), 5.21 (bd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.16 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-3'), 5.00 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-3), 4.71 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.68 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.21 (bdd, 1 H, $J_{5',6'} = J_{5',6a'} = 6.7$ Hz, H-5'), 3.85 (ddd, 1 H, $J_{5,6}$ 1.8, $J_{5,6'}$ 4.2 Hz, H-5), 3.72 (dd, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 2.08, 2.06, 2.02, 1.99, 1.98, 1.94, 1.79 (7s, 21 H, 7 CH_3CO). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Calcd for $C_{44}H_{52}O_{21}N_2$: C, 55.93; H, 5.55; N, 2.96. Found: C, 55.78; H, 5.40; N, 2.91.

N-(9-Fluorenylmethoxycarbonyl)-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl]-L-serine (**27**) (*Method A*).—Amorphous solid, 93%, $[\alpha]_D$ +35.1° (*c* 0.5 chloroform), R_f 0.47 (toluene–ethanol 8:2). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Calcd for $C_{44}H_{52}O_{21}N_2$: C, 55.93; H, 5.55; N, 2.96. Found: C, 56.21; H, 5.39; N, 2.74.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonine (**28**) (*Method A*).—Amorphous solid, 92%, $[\alpha]_D$ +59° (*c* 0.5 chloroform), R_f 0.55 (dichloromethane–methanol 85:15); lit [27]. $[\alpha]_D$ +65.0° (*c* 1.45, chloroform). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Calcd for $C_{33}H_{38}O_{13}N_2$: C, 59.10; H, 5.71; N, 4.18. Found: C, 59.34; H, 5.60; N, 4.05.

N-(9-Fluorenylmethoxycarbonyl)-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-L-serine (**41**).—Amorphous solid, 95%, $[\alpha]_D$ +12.7° (*c* 0.8 chloroform), R_f 0.5 (dichloromethane–methanol 85:15). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Calcd for $C_{44}H_{52}O_{21}N_2$: C, 55.93; H, 5.55; N, 2.96. Found: C, 55.99; H, 5.42; N, 2.73.

N-(9-Fluorenylmethoxycarbonyl)-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl]-L-serine (**26**) *(direct route from Schiff base ester* **11** *without purification of intermediates).*—Compound **11** (1.38 g) was dissolved in tetrahydrofuran (13.5 mL), and trifluoroacetic acid (1.5 mL) and water (0.3 mL) were added. Within 10 min the hydrolysis was complete. The solution was diluted with dichloromethane (150 mL), washed with saturated $NaHCO_3$ (3 × 15 mL), water (3 × 15 mL), dried (Na_2SO_4), and evaporated in vacuo. The syrup was dissolved in dichloromethane (10 mL) and treated with 9-fluorenylmethyl chloroformate (373 mg) in the presence of triethylamine (0.5 mL) for 50 min at room temperature. (The reaction was followed by TLC, toluene–ethylacetate 6:4.) The solution was then diluted with dichloromethane (150 mL), washed with saturated $NaHCO_3$ solution (3 × 15 mL), water (3 × 15 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was dissolved in methanol (300 mL) and water (30 mL), and 5% palladium on activated carbon (200 mg) was added. This mixture was vigorously stirred under H_2 (1 atm) for 2 h (followed by TLC). The Pd/C was filtered from the reaction, and the solution evaporated in vacuo. The residue was stirred in dioxane (25 mL) in the presence of acetic anhydride (0.2 mL) and Et_3N (0.6 mL) for 30 min at room temperature (followed by TLC). The mixture was concentrated in vacuo, and dissolved in dichloromethane (200 mL). The acidity of the solution was adjusted to pH 3 with acetic acid, and washed with water (3 × 25 mL), dried ($MgSO_4$), evaporated, then re-evaporated from dry toluene in vacuo. The residue was separated on a short column to give **26** in 88% overall yield. Amorphous solid, $[\alpha]_D$ +54.8° (*c* 1.0 chloroform), R_f 0.5 (dichloromethane–methanol 85:15). Anal. Found: C, 56.08; H, 5.67; N, 2.81.

Preparation of 2-acetamido-Schiff base glycosides **30–32**. *The Staudinger route.*—2-Azido–Schiff base glycosides (**9–12**) (0.1 mmol), triphenylphosphine (1.3 equiv), and acetic anhydride (1.5 equiv) were stirred in dry tetrahydrofuran (3 mL) for 16 h at room temperature. Into this mixture 1 M ammonium acetate in water (0.2 mL) was added, and the reaction was stirred for another 2–5 days (followed by TLC). The reaction mixture was evaporated, then re-evaporated from dry toluene in vacuo. The residue was dissolved in dichloromethane (30 mL), washed with water (3 × 5 mL), evaporated and separated on a column to give **30–32**.

N-Diphenylmethylene-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine benzyl ester (**30**).—Amorphous solid, 68%, $[\alpha]_D$ +25° (*c* 0.18, chloroform), R_f 0.45 (dichloromethane–acetone 87:13). ^{1}H- and ^{13}C-NMR data are provided in Tables 1–6. Anal. Calcd for $C_{37}H_{40}O_{11}N_2$: C, 64.53; H, 5.85; N, 4.07. Found: C, 64.31; H, 5.92; N, 4.18.

N-Diphenylmethylene-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl]-L-serine benzyl ester (**31**).—Foam, 77%, $[\alpha]_D$ −17° (*c* 1.0, chloroform), R_f 0.37 (hexane–ethylacetate–methanol 5:5:1). Characteristic ^{1}H-NMR data : δ 7.78–7.11 (m, 15 H, arom.), 5.63 (d, 1 H, NH), 5.31 (dd, 1 H, $J_{3'-4'}$ 3.3 Hz, $J_{4'-5'}$ 1 Hz, H-4'), 4.93 (dd, 1 H, $J_{2'-3'}$ 10.3 Hz, H-3'), 4.71 (d, 1 H, J_{1-2} 3.5 Hz, H-1), 4.47 (d, 1 H, $J_{1'-2'}$ 7.8 Hz, H-1'), 2.13–1.95 (7s, 21 H, 7 CH_3CO). Anal. Calcd for $C_{45}H_{56}O_{19}N_2$: C, 60.24; H, 5.78; N, 2.87. Found: C, 60.01; H, 5.80; N, 2.65.

N-Diphenylmethylene-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl]-L-serine benzyl ester (**32**). —Foam, 61%, $[\alpha]_D$ +27° (*c* 0.74, chloroform), R_f 0.45 (hexane–ethylacetate–methanol 5:5:1). ^{1}H-NMR characteristic data : δ 7.70–7.14 (m, 15 H, arom.), 5.45 (d, 1 H, J_{NH-2} 9.5 Hz, NH), 5.32 (dd, 1 H, $J_{3'-4'}$ 3.3 Hz, $J_{4'-5'}$ 1 Hz, H-4'), 5.15 (s, 2 H, CH_2Ph), 5.22 (dd, 1 H, J_{2-3} 4.3 Hz, J_{3-4} 8.8 Hz, H-3), 5.12 (dd, 1 H, $J_{1'-2'}$ 7.9 Hz, $J_{2'-3'}$ 10.5 Hz, H-2') 4.95 (dd, 1 H, $J_{2'-3'}$ 10.5 Hz, $J_{3'-4'}$ 3.3 Hz, H-3'), 4.70 (d, 1 H, J_{1-2} 1.6 Hz, H-1), 4.53 (d, 1 H, $J_{1'-2'}$ 7.9 Hz, H-1'), 4.40 (dd, 1 H, $J_{\alpha-b}$ 8.9 Hz, $J_{\alpha-b'}$ 4.4 Hz, H-a) 2.18–1.95 (7s, 21 H, 7 CH_3CO). Anal. Calcd for $C_{49}H_{55}O_{19}N_2$: C, 60.30; H, 5.68; N, 2.87. Found: C, 60.33; H, 5.94; N, 2.55.

O-(3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (**34**).— Compound **30** (230 mg) and 5% Pd/C (200 mg) were stirred in methanol (60 mL) under H_2 (1 atm) for 2 h. The Pd/C was filtered, and the solution evaporated in vacuo, dissolved in water (100 mL), and washed with toluene (3 × 15 mL). The inorganic phase was lyophilized to give the amorphous **34**. 135 mg (93%), $[\alpha]_D$ +95° (c 0.3, c water), R_f 0.4 (dichloromethane–methanol 65:35). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Calcd for $C_{17}H_{26}O_{11}N_2$: C, 47.00; H, 6.03; N, 6.45. Found: C, 47.21; H, 5.87; N, 6.33.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine (**37**).—Compound **34** (100 mg) was stirred in a mixture of 10% $NaHCO_3$ in water (10 mL) and dioxane (6 mL) at 0°C, and Fmoc-Cl (60 mg, 1 equiv) in dioxane (6 mL) was added over 30 min. After stirring at 0°C for 4 h and then at room temperature for 8 h, the reaction mixture was adjusted to pH 4 with 10% HCl, evaporated, dissolved in dichloromethane (100 mL), washed with water (3 × 15 mL), dried, evaporated, and chromatographed on SiO_2 to give **37**. Amorphous solid,133 mg (88%), $[\alpha]_D$ +87.5° (*c* 2, chloroform), R_f 0.4 (dichloromethane–methanol 85:15); lit. [27] $[\alpha]_D$ +89.9° (*c* 1.0, chloroform.)

N-9-Fluorenylmethoxycarbonyl-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-serine (**24**).—The Staudinger route as in **9** → **30** was followed, using **8** as starting material. The product was separated on a short SiO_2 column with dichloromethane–acetone 9:1 (R_f 0.35) to give **29**. Without characterization the evaporated residue was hydrogenated and treated with Fmoc-Cl (cf. **43** → **45**, below) to give **24**, in a 47% overall yield. Anal. Found: C, 58.41; H, 5.64; N, 4.24.

N-Diphenylmethylene-O-(3,4,6-tri-O-acetyl-2-deoxy-2-{[(2,2,2-trichloroethoxy)-carbonyl]amino}-β-D-glucopyranosyl)-L-serine benzyl ester (**43**).—Glycosylation as in **6** → **38** (method B), using bromide **42** [25] (1.5 equiv) and silver triflate (1.5 equiv) to provide **43**. Foam, 83%, $[\alpha]_D$ −11.3° (*c* 0.8, chloroform), R_f 0.6 (hexane–ethylacetate 1:1). ^{1}H-NMR data are provided in Tables 1–4. Anal. Calcd for $C_{38}H_{39}O_{12}N_2Cl_3$: C, 55.52; H, 4.78; N, 3.41; Cl, 12.94. Found: C, 55.76; H, 4.81; N, 3.55; Cl, 13.05.

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-{[(2,2,2-tri-chloroethoxy)carbonyl]amino}-β-D-glucopyranosyl)-L-serine (**45**).—Schiff base **43** (586 mg) and 5% Pd/C (200 mg) were stirred in methanol (300 mL) under H_2 (1 atm) for 3 h. The Pd/C was filtered off, the solvent evaporated in vacuo, and the residue was dissolved in water (250 mL) and washed with toluene (3 × 50 mL). The inorganic phase was evaporated to give **44**. Without any purification the amino acid glycoside was stirred in a mixture of 10% $NaHCO_3$ in water (20 mL) and dioxane (12 mL) at 0°C, and Fmoc-Cl (259 mg, 1 equiv) in dioxane (10 mL) was added over 30 min. After stirring at 0°C for 4 h, and then at room temperature for 8 h, the pH was set to 4 with 10% HCl. The reaction mixture was evaporated, dissolved in dichloromethane (150 mL), washed with water (3 × 25 mL), dried, evaporated, and chromatographed to give **45** as a foam. 575 mg (73%), $[\alpha]_D$ +17.9° (*c* 0.56, chloroform), R_f 0.2 (dichloromethane–methanol 9:1). Characteristic ^{1}H-NMR data : δ 7.76–7.27 (m, 8 H, arom.), 5.90 (d, 1 H, *J* 8.2 Hz, NH), 5.43 (d, 1 H, *J* 7.8 Hz, NH), 5.23 (dd, $J_{2-3} = J_{3-4} = 10.2$ Hz, H-3), 5.03 (dd, 1 H, J_{4-5} 10.0 Hz, H-4), 4.65 (d, 1 H, J_{1-2} 8.3 Hz, H-1), 3.63 (m, 2 H, H-2 and H-5), 2.06, 2.00, 1.98 (3s, 9 H, 3 CH_3CO). FAB-MS: Calcd for $C_{33}H_{35}O_{14}N_2Cl_3$ 788.1, found *m/z* 789.1 (MH^+).

N-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine (**46**).—Compound **45** (0.8 g) was dissolved in acetic acid (20 mL) and Zn (dust, 1 g) was added. The mixture was stirred for 4 h at room temperature, filtered through Celite, and evaporated from toluene in vacuo. The residue was dissolved in dichloromethane (30 mL) and acetic anhydride (0.11 mL), and Et_3N (0.2 mL) were added. After 2 h, the solution was diluted with dichloromethane (100 mL), washed with water (3 × 10 mL) dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography provided **46** as a oil. 415 mg (62%), $[\alpha]_D$ −21.9° (*c* 1.7, chloroform), R_f 0.2 (dichloromethane–methanol 9:1). ^{1}H-NMR data are provided in Tables 1–4. Anal. Calcd for $C_{32}H_{36}O_{13}N_2$: C, 58.53; H, 5.53; N, 4.27. Found: C, 58.58; H, 5.42; N, 4.10.

Fmoc glycosides (**26** *and* **27**) *from 2-acetamido-glycosides* (**31** *and* **32**).—Compounds **31–32** were treated as in **43** → **45** above, to provide **26** and **27**.

N-(9-Fluorenylmethoxycarbonyl)-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl]-L-serine (**26**).—Amorphous solid, 90%, $[\alpha]_D$ +56.1° (*c* 0.5 chloroform), R_f 0.5 (dichloromethane–methanol 85:15). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Found: C, 55.84; H, 5.69; N, 2.82.

N-(9-Fluorenylmethoxycarbonyl)-O-[O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-3,6-di-O-acetyl-2-acetamido-2-deoxy-α-D-mannopyranosyl]-L-serine (**27**). —Amorphous solid, 91%, $[\alpha]_D$ +29.4° (*c* 0.8 chloroform), R_f 0.44 (dichloromethane–methanol 85:15). ^{13}C-NMR data are provided in Tables 5 and 6. Anal. Found: C, 55.74; H, 5.67; N, 2.82.

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