Carbohydrate Research, 196 (1990) 75–93
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF 2-ACETAMIDO-2-DEOXYGLUCOSYLASPARAGINE GLYCO-TRIPEPTIDES AND -PENTAPEPTIDES BY SELECTIVE C- AND N-TERMINAL ELONGATION OF THE PEPTIDE CHAIN

HERBERT WALDMANN, JOACHIM MÄRZ, AND HORST KUNZ*
Institut für Organische Chemie, Johannes Gutenberg Universität Mainz, Becherweg 18-20, D-6500 Mainz (F.R.G.)

(Received February 14th, 1989; accepted for publication, July 10th, 1989)

ABSTRACT

The synthesis of protected 2-acetamido-2-deoxyglucosylasparagine glycopeptides, using the allyl ester as the C-terminal protecting group, their deprotection, and some possible applications of these glycopeptides for the synthesis of modified silica gels and the construction of liposomes are described. The selective carboxyl deblocking is achieved under neutral conditions by rhodium(I)-catalyzed isomerization of the allyl group followed by hydrolysis of the resulting propenyl ester. The *tert*-butoxycarbonyl group can be cleaved selectively in the presence of the allyl ester with hydrogen chloride in ether. The allyl ester and the acetates can be removed simultaneously with ammonia in methanol. This method opens up a preparative route to glycopeptide model structures of biological interest.

INTRODUCTION

Most of the proteins in eukaryotic cells are glycosylated and these glycoproteins play central roles in such biological processes as recognition on cell membranes, intercellular communication, and tumor growth[1]. In contrast to peptides, glycopeptides representing partial structures of glycoproteins are not easily available from gene-technological procedures. The central problem in glycopeptide synthesis is the selective protection and deprotection of numerous functional groups. The acid-labile glycosidic bond and the base-labile O-glycosylic bond in serine- and threonine-O-glycopeptides require the use of protecting groups which can be removed under almost neutral conditions[2]. We have shown that the use of the allyl ester for C-terminal protection of amino acids is particularly advantageous for glycopeptide synthesis[3–5]. This group can be cleaved by palladium(0)-catalysed allyl transfer to weakly basic or neutral nucleophiles and rhodium(I)-catalyzed isomerization followed by hydrolysis of the resulting propenyl ester. We now describe the application of this methodology in combina-

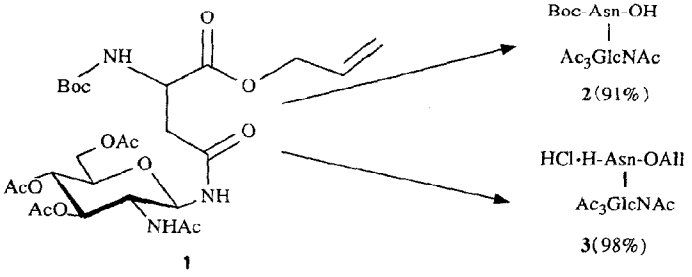
*Author for correspondence.

0008-6215/90/$ 03.50

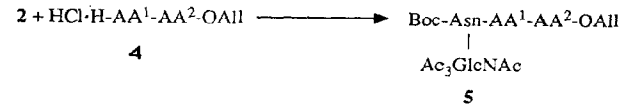
tion with acid hydrolysis of the *tert*-butoxycarbonyl (Boc) group in the construction of complex N-glyco-tri- and -penta-peptides.

RESULTS AND DISCUSSION

The key intermediate was the fully protected 2-acetamido-2-deoxyglucosyl-asparagine derivative **1**, which has been used[3] in the preparation of the glycopeptide partial sequence A^{80}–A^{84} of human fibroblast interferon, namely, H-Asn(GlcNAcβ-1)-Glu-Thr-Ile-Val-OH[3]. Compound **1** was obtained[4] as follows. The ester obtained by alkylation of the copper–potassium complex of aspartic acid with phenacyl bromide was acylated with *tert*-butoxycarbonyl azide and the α-carboxyl group was alkylated with allyl bromide. The phenacyl ester was then cleaved with thiophenolate and the product was coupled to 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)[6a] to give **1**. The allyl ester was removed from **1** by the Wilkinson-catalyst $(PPh_3)_3Rh(I)Cl$ to give **2** in high yield. Alternatively, the Boc-group could be cleaved selectively and quantitatively from **1** using hydrogen chloride in dichloromethane; the allyl ester was not affected under these acidic conditions. Anomerization of the N-glucosylic bond was not observed.



1



AA = amino acid

PG = protecting group

Glycotripeptides **5** were accessible from **2** by C-terminal chain elongation with the dipeptide allyl ester hydrochlorides **4**[4,5]. These derivatives were synthesized

TABLE I

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF THE DIPEPTIDE ALLYL ESTER HYDROCHLORIDES **4**

Compound	*AA¹–AA²*	*Yield (%)*	*M.p. (°)*	*$[\alpha]_D^{22}$ (°) (c 1, MeOH)*	*Formula*	*Elemental analysis*	*C*	*H*	*N*
4a	Ala–Thr	97	96–97	−9.6	$C_{10}H_{19}ClN_2O_4$	Calc.	45.03	7.18	10.50
						Found	45.80	7.15	10.19
4b	Ile–Ser	88	162–164 (dec.)	+5.9	$C_{12}H_{23}ClN_2O_4$	Calc.	48.90	7.86	9.50
						Found	48.74	7.75	9.33

TABLE II

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF THE *N*-ACYLGLYCOTRIPEPTIDE ALLYL ESTERS **5**

Compound	*AA¹–AA²*	*Yield (%)*	*M.p. (°)*	*$[\alpha]_D^{22}$ (°) (c, MeOH)*	*Formula*	*Elemental analysis*	*C*	*H*	*N*
5a	Ala–Thr	87	221 (dec.)	−15.2 (0.8)	$C_{33}H_{51}N_5O_{16}$	Calc.	51.22	6.64	9.05
						Found	50.88	6.62	9.21
5b	Leu–Phe	83	237 (dec.)	−25.5 (1)	$C_{41}H_{59}N_5O_{15}$	Calc.	57.13	6.90	8.12
						Found	57.43	7.19	8.25
5c	Ile–Leu	85	254 (dec.)	−32.8 (1)	$C_{38}H_{61}N_5O_{15} \cdot 0.5\ H_2O$	Calc.	54.53	7.47	8.37
						Found	54.54	7.33	8.12
5d	Thr–Ser	86	210–211 (dec.)	−12.0 (1)	$C_{13}H_{51}N_5O_{17}$	Calc.	50.05	6.75	8.84
						Found	49.72	6.40	8.93

via the N-terminal deprotection of Boc-dipeptide allyl ester (see Tables I and II).

The hydrochloride **3** could be used for the condensation with the N-protected dipeptides **6** (see Table III) to give the fully protected glycotripeptides **7** by means of the EEDQ-method (see Table IV).

Surprisingly, the reaction of **3** with **6a** gave only 18% of **7b**, and **3** was mainly (66%) acylated by EEDQ to form the ethoxycarbonylasparagine derivative **12**. However, the use of carbodi-imide–hydroxybenzotriazole[6b] gave 51% of **7b**.

Rh(I)-catalyzed removal of the allyl esters from the glycotripeptides **5** and **7** gave the selectively carboxy-deblocked products **8** and **9** in high yields, which were

TABLE III

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF THE *N*-ACYLDIPEPTIDES **6**

Compound	*AA¹–AA²*	*Yield (%)*	*M.p. (°)*	*$[\alpha]_D^{22}$ (°) (c, MeOH)*	*Formula*	*Elemental analysis*	*C*	*H*	*N*
6a	Boc-Leu–Thr	81	amorph.	−18.7 (1)	$C_{15}H_{28}N_2O_6$		—	—	—
							—	—	—
6b	Bz-Gly–Thr	95	170–172 (dec.)	+8.6 (0.5)	$C_{13}H_{16}N_2O_5$	Calc.	55.71	5.75	9.99
						Found	55.14	5.96	9.58

TABLE IV

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF THE *N*-ACYLGLYCOTRIPEPTIDE ALLYL ESTERS **7**

Compound	*AA¹–AA²*	*Yield (%)*	*M.p. (°)*	*$[\alpha]_D^{22}$ (°) (c, MeOH)*	*Formula*	*Elemental analysis*	*C*	*H*	*N*
7a	Boc-Leu–Thr	78	206 (dec.)	−5.9 (1)	$C_{36}H_{57}N_5O_{16} \cdot H_2O$	Calc.	51.85	7.13	8.40
						Found	51.68	7.04	8.54
7b	Bz-Gly–Thr	18[a] 51[b]	221	−4.3 (0.5)	$C_{34}H_{45}N_5O_{15} \cdot H_2O$	Calc.	52.24	6.06	8.96
						Found	51.96	5.96	9.31

[a]N-terminal elongation with EEDQ[6a]. [b]N-terminal elongation with DCC–HOBt[6b].

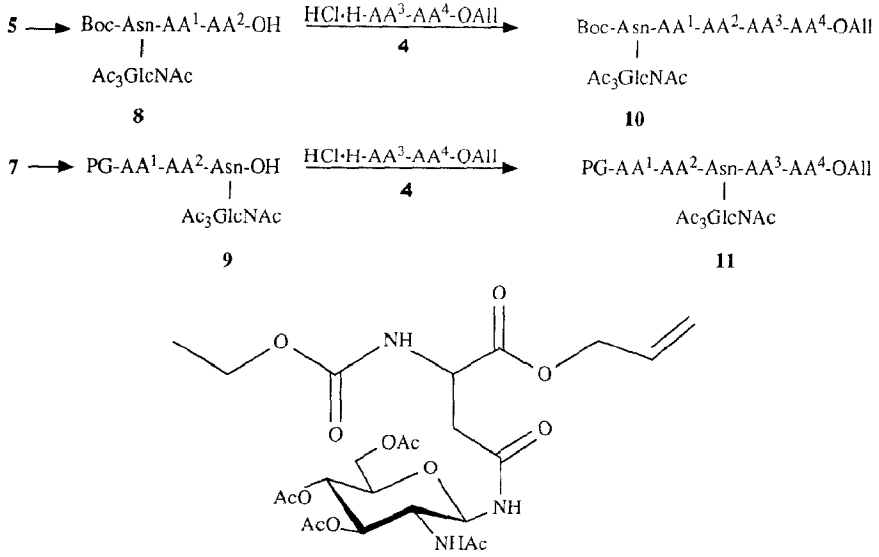


TABLE V

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF THE *N*-ACYLGLYCOTRIPEPTIDES **8** AND **9**

Compound	*AA¹–AA²*	*Yield (%)*	*M.p. (°)*	*$[\alpha]_D^{22}$ (°) (c, solvent)*	*Formula*	*Elemental analysis*	*C*	*H*	*N*
8	Ala–Thr	98	205–206 (dec.)	−11.8 (1, MeOH)	$C_{30}H_{47}N_5O_{16}$	Calc.	49.11	6.46	9.55
						Found	49.55	6.93	9.00
9a	Boc-Leu–Thr	93	180–182 (dec.)	+2.7 (1, Me_2SO)	$C_{33}H_{53}N_5O_{16}$	Calc.	51.09	6.89	9.03
						Found	51.40	6.78	8.94
9b	Bz-Gly–Thr	93	189–192	−1.6 (0.3, MeOH)	$C_{31}H_{41}N_5O_{15}$	Calc.	51.45	5.71	9.68
						Found	51.94	6.01	9.71

TABLE VI

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF THE *N*-ACYLGLYCOPENTAPEPTIDE ALLYL ESTERS **10** AND **11**

Compound	*AA^1–AA^2*	*AA^3–AA^4*	*Yield (%)*	*M.p. (°)*	*$[\alpha]_D^{22}$ (°) (c, solvent)*	*Formula*	*Elemental analysis*	*C*	*H*	*N*
10	Ala–Thr	Ile–Leu	83	268–270 (dec.)	−31.0 (1, MeOH)	$C_{45}H_{73}N_7O_{18}\cdot 3\,H_2O$	Calc.	51.27	7.55	9.30
							Found	51.54	7.02	9.21
11a	Boc-Leu–Thr	Ala	83	207 (dec.)	−22.2 (1, MeOH)	$C_{43}H_{69}N_7O_{19}\cdot 2\,H_2O$	Calc.	50.43	7.18	9.57
							Found	50.32	7.06	9.09
11b	Bz-Gly–Thr	Ile–Ser	81	241–243 (dec.)	−17.1 (0.5, Me_2SO)	$C_{43}H_{61}N_7O_{18}$	Calc.	53.58	6.38	10.17
							Found	52.96	6.65	9.93
12			66	268–270	+1.8 (1, MeOH)	$C_{24}H_{35}N_3O_{13}$	Calc.	50.25	6.15	7.32
							Found	50.43	6.13	7.39

TABLE VII

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF THE *N*-GLYCOPENTAPEPTIDE AMIDES **13**, **14**, AND **15**

Compound	*Yield (%)*	*M.p. (°)*	*$[\alpha]_D^{22}$ (°) (c, solvent)*	*Formula*	*Elemental analysis*	*C*	*H*	*N*
13	82	201 (dec.)	−6.6 (0.37, H_2O)	$C_{24}H_{42}N_6O_{13}\cdot 2\,H_2O$	Calc.	43.77	7.04	12.76
					Found	43.76	6.52	12.19
14	90	254–256 (dec.)	−20.1 (0.1, H_2O–2-propanol 1:2)	$C_{36}H_{64}N_8O_{14}\cdot 2\,H_2O$	Calc.	49.76	7.89	12.90
					Found	50.03	7.68	12.70
15	86	260–262	−26.6 (0.24, H_2O)	$C_{34}H_{52}N_8O_{14}$	Calc.	51.25	6.58	14.06
					Found	50.71	7.19	13.64

elongated with the dipeptide allyl ester hydrochlorides **4** to give the 2-acetamido-2-deoxyglucosylasparaginyl pentapeptides **10** and **11**, again using EEDQ.

The glycopeptides in Table VI represent partial sequences of naturally occurring glycoproteins: **10** is derived from human α_1-acid glycoprotein[7], **11a** from human orosomucoid[8], and **11b** contains the linkage region of turkey ovomucoid[9].

The structures of these compounds were confirmed by 2D-n.m.r. spectroscopy. In Figs. 1 and 2, the COSY- and the ^{13}C–^{1}H-correlated spectra of the glycopeptide **10** are shown; all ^{1}H and ^{13}C resonances of **10** could be assigned unambiguously.

For the further use of the glycopeptides synthesized in biochemical or immunological investigations, it was necessary to remove all protecting groups. The fully protected glycopeptides **4d**, **10**, and **11b** were each treated with methanolic ammonia, and all *O*-acetates were cleaved and the allyl ester was removed to give the peptide amides **13**–**15**, respectively, in high yields.

The glycopeptide amides **13**–**15** were each treated with 3M HCl to remove the Boc-group quantitatively, to yield totally deblocked 2-acetamido-2-deoxyglucosyl-

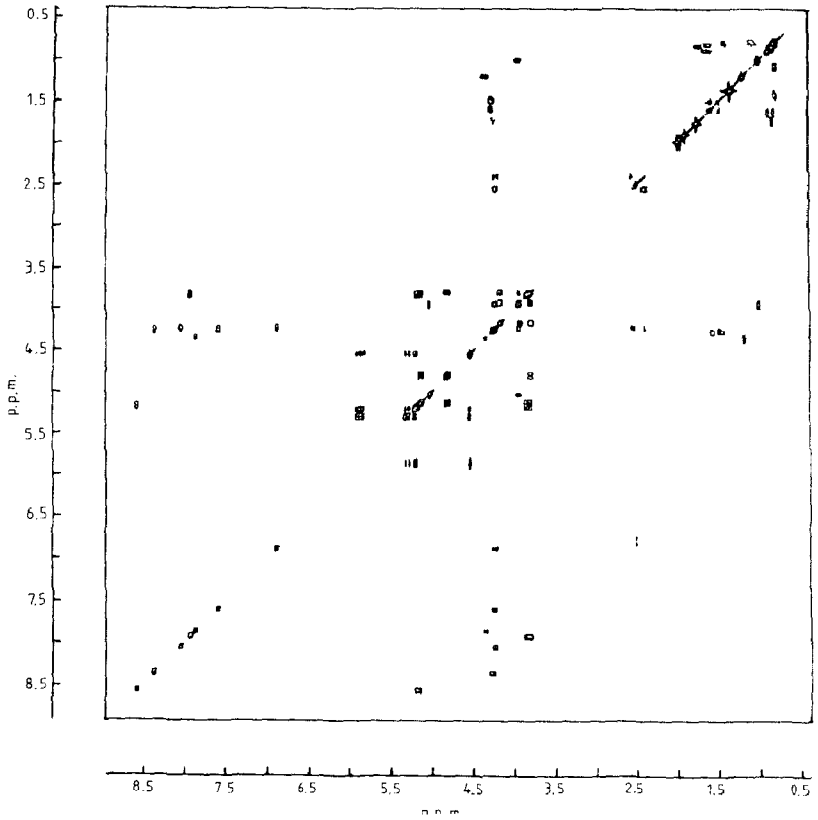


Fig. 1. COSY-spectrum of Boc-Asn(Ac_3GlcNAc)-Ala-Thr-Ile-Leu-OA11 (**10**).

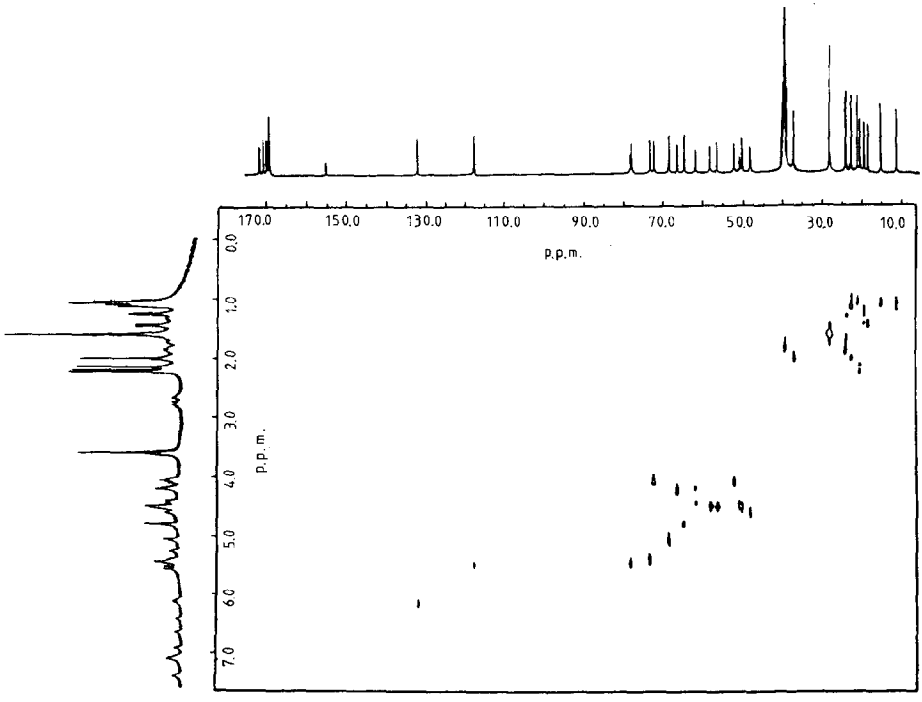
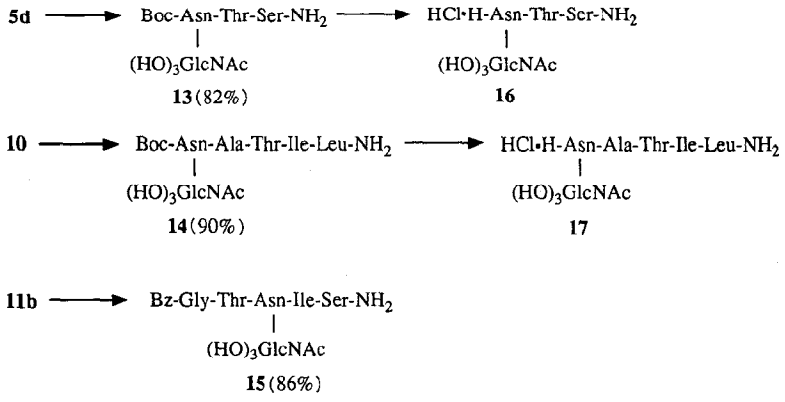


Fig. 2. ^{13}C–^{1}H-correlated spectrum of Boc-Asn(Ac_3GlcNAc)-Ala-Thr-Ile-Leu-OA11 (**10**).



pentapeptide amides **16** and **17**. Under these conditions, the N-glycosylic linkage was not affected.

Chemically synthesized glycopeptides can serve not only as starting materials for biochemical and immunological studies but can also be linked to silica gel to provide materials for affinity chromatography, for the construction of amphiphilic derivatives which permit the formation of liposomes containing glycopeptide

structures exposed on their surface. These supramolecular assemblies may be considered as models for cell membrane structures.

Preparation of modified silica gels for affinity-h.p.l.c. — The *N*-glycosylasparagine **2** was reacted with 3-(triethoxysilyl)propylamine, using the EEDQ method, and with 3-(triethoxysilyl)propyl isocyanate[10] to yield the amide **18**.

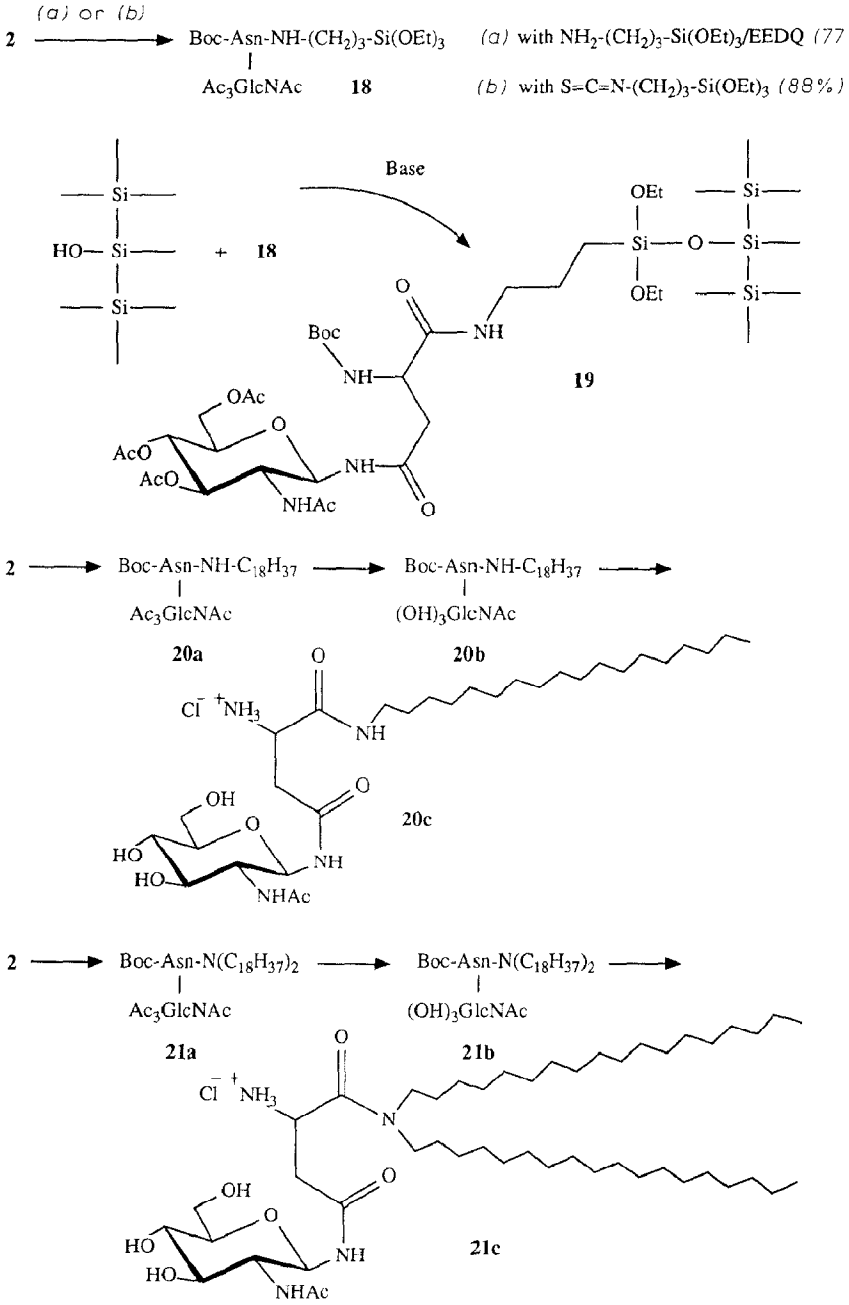


TABLE VIII

YIELDS, PHYSICAL DATA, AND ELEMENTAL ANALYSIS OF COMPOUNDS **20a**, **20b**, **20c**, **21a**, **21b**, AND **21c**

Compound	*Yield (%)*	*M.p.* (°)	$[\alpha]_D^{22}$ (°) (c, *solvent*)	*Formula*	*Elemental analysis*			
						C	*H*	*N*
20a	99	—	−7.8 (0.5, CH_2Cl_2)	$C_{41}H_{72}N_4O_{12}$	Calc.	60.57	8.93	6.89
					Found	59.83	8.83	6.80
20b	quant.	244 (dec.)	+15.6 (0.5, $CHCl_3$–MeOH 1:1)	$C_{35}H_{66}N_4O_9 \cdot H_2O$	Calc.	59.63	9.72	7.95
					Found	59.80	9.73	7.94
20c	quant.	208	40.4 (0.2, hexane–AcOH–EtOH 16:2:3)	$C_{30}H_{59}ClN_4O_7$	Calc.	57.81	9.54	8.99
					Found	57.54	9.36	8.75
21a	52	122–123	−14.7 (0.5, $CDCl_3$)	$C_{59}H_{110}N_4O_{12}$	Calc.	66.38	10.39	5.25
					Found	66.15	9.89	5.34
21b	91	81–82	+4.6 (0.5, $CHCl_3$)	$C_{53}H_{104}N_4O_9 \cdot H_2O$	Calc.	66.34	11.14	5.84
					Found	66.65	10.76	6.57
21c	quant.	205	+9.3 (0.2, CH_2Cl_2)	$C_{48}H_{97}ClN_4O_7 \cdot 1.5\ H_2O$	Calc.	63.72	11.14	6.19
					Found	63.72	10.52	6.33

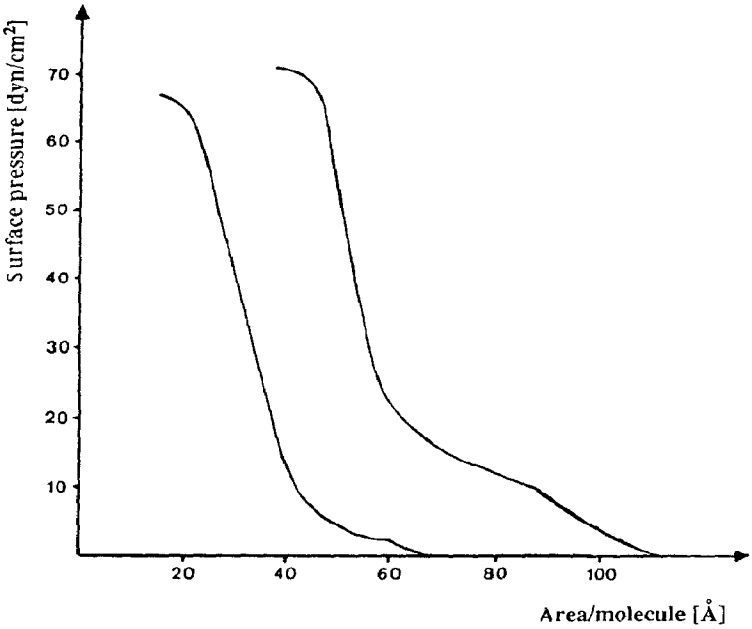


Fig. 3. Pressure–surface diagrams of **20c** and **21c**.

In the presence of weak bases, the product **18** could be connected to silica gel[11] (**19**). The capacity of the product was 2.2 μmol/m^2. The *O*-acetates then could be removed with hydrazine or ammonia in methanol, and the modified silica gels can be used for the isolation of lectins by affinity-h.p.l.c.

Preparation of artificial liposomes. — Glycopeptides can be modified to give amphiphilic compounds with a hydrophilic carbohydrate-containing head and a hydrophobic soapy tail which can be applied in the construction of lipid bilayer membranes[12]. For this purpose, the 2-acetamido-2-deoxyglucosylasparagine **2** was condensed with octadecyl- or dioctadecyl-amine using the EEDQ-procedure. The protecting groups were then removed to obtain the soapy compounds **20c** and **21c**.

Amphiphilic compounds like **20c** and **21c** form monomolecular layers[13]. Using a film balance, it is possible to measure the tension difference between water and amphiphile-containing water in a "pressure–surface diagram". The surface which is available for the molecules is reduced continuously, and the resulting pressure against a stationary barrier is registered. The pressure rises until the highest possible density of packing of the molecules on the surface is reached. Beyond this point, the monomolecular layer collapses. Such characteristic "pressure–surface diagrams" for **20c** and **21c** are shown in Fig. 3, which prove the amphiphilic character of these compounds.

The collapsing point of **20c** is reached at 63 dyn/cm^2 which accords to a surface of 22.4 Å^2/molecule; for **21c**, the values are 68 dyn/cm^2 and 44.4

$Å^2$/molecule. From the amphiphilic conjugates, liposomes could be prepared. Thus, **21c** was suspended in water and ultrasonicated for 8 min. The resulting liposomes were isolated by gel filtration. Electron microscopy revealed spherical particles.

EXPERIMENTAL

General methods. — N.m.r.-spectra were recorded with Jeol JNM-60 (60 MHz, ^1H), Bruker WP 80 (80 MHz, ^1H; 20.15 MHz, ^{13}C), WH 90 (90 MHz, ^1H; 22.63 MHz, ^{13}C), WH 270 (270 MHz, ^1H), AM 400 (400 MHz, ^1H), and Nicolet-470 (470 MHz, ^1H) spectrometers. I.r. spectra were recorded with a Beckman Acculab-2 and optical rotations with a Perkin–Elmer Polarimeter 241. All melting points are uncorrected. T.l.c. was conducted on Silica Gel 60 F_{254} (Merck) and detection with 0.3% ninhydrin in methanol–acetic acid (97:3) or with 0.1% 1,3-dihydroxynaphthalene in ethanol–M H_2SO_4 (1:1) and heating. Only L-amino acids were used.

N^4-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosy)-N^2-(tert-butoxycarbonyl)-L-asparagine allyl ester hydrochloride (**3**). — A solution of **1** (2.4 g, 4 mmol) in dichloromethane (10 mL) was stirred with cold saturated ethereal HCl (30 mL) at room temperature. White crystals began to precipitate and, after 3 h, the solution was concentrated to dryness, the residue was triturated with dichloromethane (20 mL) and ether (30 mL), and the crystals were collected. Compound **3** (2.1 g, 98%) had m.p. 207° (dec.), $[\alpha]_D^{22}$ +18.7° (*c* 1.2, methanol); ν_{max}^{KBr} 1745 (C=O, ester) and 1665 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (75 MHz), δ 170.38, 170.02, 169.84, 169.62, 169.32, 168.59 (6 C=O), 132.02 ($CH=CH_2$), 118.13 ($CH=CH_2$), 78.12 (C-1), 73.34 (C-3), 72.61 (C-5), 68.71 (C-2), 66.14 ($OCH_2CH=CH_2$), 62.1 (C-6), 52.69 (C-4), 48.71 (α-CH), 35.0 (β-CH_2), 22.94 (CH_3CONH), 20.81, 20.80, 20.67 (3 CH_3COO).

Anal. Calc. for $C_{21}H_{32}ClN_3O_{11}$: C, 46.89; H, 6.00; N, 7.81. Found: C, 46.81; H, 6.00; N, 8.12.

Dipeptide allyl ester hydrochlorides **4**. — The yields and physical data are given in Table I. These compounds were prepared following the procedure described in ref. 4.

L-Alanyl–L-threonine (**4a**) and L-isoleucyl–L-serine (**4b**) allyl ester hydrochlorides have i.r. bands at 1730–1750 (C=O, ester) and 1660–1670 cm^{-1} (Amide I). The 90-MHz ^1H-n.m.r. spectra contained signals typical of the amino acids together with those for the allylic system at δ 5.8 (m, 1 H, $CH=CH_2$), 5.15 (dd, 1 H, J_{trans} 17, J_{gem} 1 Hz, $CH=CH_{2trans}$), 5.0 (dd, 1 H, J_{cis} 10, J_{gem} *1*, ^4J ~1 Hz, $CH=CH_{2cis}$), 4.5 (d, 2 H, *J* 5 Hz, $CH_2CH=CH_2$).

N-Acylglycotripeptide allyl esters **5**. — To a solution of **2** (0.6 g, 1 mmol), **4** (1 mmol), and triethylamine (0.1 g, 1 mmol) in dichloromethane (10 mL) was added EEDQ (0.4 g, 1.6 mmol), and the solution was stirred at room temperature for 24–72 h. The reaction was monitored by t.l.c. (chloroform–methanol). The solution was extracted with 0.5M HCl (3 × 10 mL), 0.25M Na_2CO_3 (3 × 10 mL), and

water (10 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was recrystallized from dichloromethane–ether or methanol–ether. The yields, physical data, and elemental analysis are given in Table I. The following glycopeptides were obtained by this procedure.

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*tert*-butoxycarbonyl)-L-asparaginyl-L-alanyl-L-threonine allyl ester (**5a**), R_F 0.53 (chloroform–methanol, 7:1); ν_{max}^{KBr} 1750 (C=O, ester), 1690 (urethane), and 1650 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (22.63 MHz), δ 172.5–169.0 (8 s, 8 C=O), 155.0 (C=O, urethane), 132.2 ($CH{=}CH_2$), 117.5 ($CH{=}CH_2$), 78.3 [$(CH_3)_3C$], 78.1 (C-1), 73.2 (C-3), 72.2 (C-5), 28.0 [$(CH_3)_3C$], 22.5 (CH_3CONH), 20.2 (CH_3COO), 20.0 (γ-CH Thr), 18.3 (β-CH_3 Ala).

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*tert*-butoxycarbonyl)-L-asparaginyl-L-leucyl-L-phenylalanine allyl ester (**5b**); ν_{max}^{KBr} 1750 (C=O, ester), 1690 (urethane), and 1650 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^1H (90 MHz), δ 8.6–8.3 (2 d, 2 H, *J* 8 and 7 Hz, 2 NH), 7.9–7.6 (2 d, *J* 8 Hz, 2 H, 2 NH), 7.3 (m, 5 H, Ph), 6.9 (d, 1 H, *J* 8 Hz, NH), 6.1–5.6 (m, 1 H, $CH{=}CH_2$), 2.0–1.9 (4 s, 12 H, 4 CH_3CO), 1.4 (s, 9 H, Me_3C), 0.85 (2 d, 6 H, CMe_2); ^{13}C (22.63 MHz), δ 172–169 (8 s, 8 C=O), 155 (C=O urethane), 136 (ipso-C, Ph), 131.2 ($CH{=}CH_2$), 129–125 (3 s, *o*-, *m*-, *p*-C, Ph), 117.7 ($CH{=}CH_2$), 78.2 [$(CH_3)_3C$], 77.8 (C-1), 72.6 (C-3), 72.0 (C-5), 61.7 (C-6), 27.9 [$(CH_3)_3C$], 21.5 [$CH(CH_3)$], 20.0 (CH_3CO).

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*tert*-butoxycarbonyl)-L-asparaginyl-L-isoleucyl-L-leucine allyl ester (**5c**); ν_{max}^{KBr} 1750 (C=O, ester), 1690 (urethane), 1655 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^1H (90 MHz), δ 8.6–8.3 (2 d, 2 H, *J* 9 Hz, 2 NH), 7.9 (d, 1 H, *J* 9 Hz, NH), 7.0 (d, 1 H, *J* 8 Hz, NH), 6.2–5.6 (m, 1 H, $CH{=}CH_2$), 4.55 (d, 2 H, *J* 5 Hz, $OCH_2CH{=}CH_2$), 2.0–1.9 (4 s, 12 H, 4 CH_3CO), 1.4 (s, 9 H, Me_3C), 1–0.7 [m, 12 H, $CH(Me)CH_2CH_3$ and CMe_2]; ^{13}C (22.63 MHz), δ 172–169 (8 s, 8 C=O), 155.1 (C=O, urethane), 132.2 ($CH{=}CH_2$), 117.8 ($CH{=}CH_2$), 78.3 [$(CH_3)_3C$], 77.8 (C-1), 73.4 (C-3), 72.2 (C-5), 67.4 (C-2), 61.9 (C-6), 28.0 [$(CH_3)_3C$], 24.1 [$CH(CH_3)_2$], 22.7 (CH_3CONH), 20.2 (CH_3COO), 14.9 [$CH(CH_3)CH_2CH_3$], 10.9 [$CH(CH_3)CH_2CH_3$].

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*tert*-butoxycarbonyl)-L-asparaginyl-L-threonyl-L-serine allyl ester (**5d**), R_F 0.43 (chloroform–methanol, 4:1); ν_{max}^{KBr} 1750 (C=O, ester) and 1650 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (22.63 MHz), δ 172–169 (8 s, 8 C=O), 155 (C=O, urethane), 132.2 ($CH{=}CH_2$), 117.5 ($CH{=}CH_2$), 78.5 [$(CH_3)_3C$], 78.0 (C-1), 73.3 (C-3), 72.3 (C-5), 28.1 [$(CH_3)_3C$], 22.5 (CH_3CONH), 20.2 (CH_3COO), 18.9 [$CH(OH)CH_3$].

N-Acyldipeptides **6**. — These compounds were prepared following the procedure described[4]. The yields and physical data are given in Table III.

N-tert-Butoxycarbonyl-L-leucyl-L-threonine (**6a**) and *N*-benzoylglycyl-L-threonine (**6b**) had ν_{max}^{KBr} 1710 (C=O, COOH), 1690 (urethane), 1660–1670 (Amide I), and 1520–1530 cm^{-1} (Amide II). The 90-MHz ^1H-n.m.r. spectra contained

signals typical of the amino acids. The singlet of the Boc-group in **6a** appears at δ 1.45 and that of the benzoyl group in **6b** at δ 7.3–8.0.

N⁴-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)*-N²-*(*N*-tert-butoxycarbonyl-*L*-leucyl-*L*-threonyl)*-*L*-asparagine allyl ester* (**7a**). — A solution of **6a** (1 g, 3 mmol), **5** (1.6 g, 3 mmol), triethylamine (0.3 g, 3 mmol), and EEDQ (1.5 g, 6 mmol) in chloroform (30 mL) was stirred at room temperature for 10 days, then extracted with 0.5M HCl (3 × 20 mL), 0.25M Na_2CO_3 (3 × 20 mL), and water (20 mL), dried ($MgSO_4$), filtered, and concentrated to dryness. The residue was recrystallized from dichloromethane–ether to give **7a** (for the yield, physical data, and elemental analysis, see Table III), R_F 0.72 (chloroform–methanol, 7:1); ν_{max}^{KBr} 1750 (C=O, ester) and 1660 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (22.63 MHz), δ 172.4–169.2 (8 s, 8 C=O), 155.3 (C=O urethane), 132.2 ($CH=CH_2$), 117.4 ($CH=CH_2$), 78.1 [$(CH_3)_3C$ and C-1], 73.3 (C-3), 72.3 (C-5), 28.0 [$(CH_3)_3C$], 24.2 [$CH(CH_3)_2$], 22.9 and 22.4 [$CH(CH_3)_2$], 21.3 (CH_3CONH), 20.4 and 20.2 (CH_3COO), 18.9 [$CH(OH)CH_3$].

N⁴-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)*-N²-*(*N*-benzoylglycyl-*L*-threonyl)*-*L*-asparagine allyl ester* (**7b**). — *(a) Modified carbodiimide procedure*[6a]. To a solution of **5** (1 g, 1.8 mmol), triethylamine (0.18 g, 1.8 mmol), 1-hydroxybenzotriazole (0.73 g, 5.4 mmol), and *N*-benzoyldipeptide **6b** (0.5 g, 1.8 mmol) in dichloromethane (30 mL) at 0° was added dicyclohexylcarbodiimide (0.55 g, 2.7 mmol). After stirring at room temperature for 7 days, the solution was concentrated *in vacuo* and the residue was purified by column chromatography (silica gel; acetone–light petroleum, 2:1). The product was recrystallized from methanol–ether.

(b) EEDQ procedure[6b]. To a solution of **6b** (2 g, 7.4 mmol), **5** (3.9 g, 7.4 mmol), and triethylamine (0.75 g, 7.4 mmol) in chloroform (100 mL) was added EEDQ (3.6 g, 15 mmol). The solution was stirred at room temperature for 7 days and then concentrated to dryness. The residue was subjected to column chromatography [light petroleum–ethyl acetate (2:1), light petroleum–acetone (2:1), and acetone]. The first fractions contained **12** (2.7 g, 66%) and the last fractions contained **7b** (for the yields, physical data, and elemental analysis, see Table III), R_F 0.17 (acetone–light petroleum, 2:1); ν_{max}^{KBr} 1750 (C=O, ester) and 1660 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^1H (90 MHz), δ 8.7 (d, 1 H, *J* 10.9 Hz, NH), 8.26 (d, 1 H, *J* 9.6 Hz, NH), 7.9–7.4 (m, 8 H, 3 NH and Ph), 6.1–5.7 (m, 1 H, $CH=CH_2$), 4.5 (d, 2 H, *J* 5 Hz, $OCH_2CH=CH_2$), 1.99, 1.96, 1.90 (3 s, 9 H, 3 CH_3COO), 1.74 (s, 3 H, CH_3CONH), 1.0 [d, 3 H, *J* 6.5 Hz, $CH(OH)CH_3$]; ^{13}C (22.63 MHz), δ 170.4–169 and 166.6 (9 C=O), 134.0 (ipso-C, Ph), 132.3 ($CH=CH_2$), 131.4 (*p*-C, Ph), 128.3 (*m*-C, Ph), 127.3 (*o*-C, Ph), 117.5 ($CH=CH_2$), 78.0 (C-1), 73.3 (C-3), 72.3 (C-5), 42.7 (α-CH_2 Gly), 22.6 (CH_3CONH), 20.4 (CH_3COO), 19.3 [$CH(OH)CH_3$].

N⁴-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)*-N²-*(ethoxycarbonyl)*-*L*-asparagine allyl ester* (**12**). — Compound **12** (2.7 g, 66%) had R_F 0.72 (acetone–light petroleum, 2:1); ν_{max}^{KBr} 1750 (C=O, ester), 1705 (urethane), and 1670

cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (22.63 MHz), δ 171.1–169.3 (6 s, C=O), 155.8 (C=O, urethane), 132.3 ($CH=CH_2$), 117.3 ($CH=CH_2$), 78.0 (C-1), 73.3 (C-3), 72.2 (C-5), 68.4 (C-2), 52.1 (C-4), 50.2 (α-CH), 22.4 (CH_3CONH), 20.3 (CH_3COO), 14.4 (CH_2CH_3).

N-*Acylglycotripeptides* **8** *and* **9**. — To a solution of **5** or **7** (1 mmol) in 9:1 ethanol–water (20 mL) at 70° was added tris(triphenylphosphine)rhodium(I) chloride (0.1 g, 0.11 mmol, 11 mol%). After 24 h, the sodium was filtered and concentrated to dryness, and the residue was recrystallized from dichloromethane–ether or methanol–ether. The product could also be isolated by chromatography on silica gel (chloroform–methanol). The yields, physical data, and elemental analysis are given in Table V. Using this procedure, the following glycotripeptides were prepared.

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*tert*-butoxycarbonyl)-L-asparaginyl-L-alanyl-L-threonine (**8**); ν_{max}^{KBr} 1750 (C=O, ester), 1685 (urethane), and 1660 cm^{-1} (Amide I). F.a.b.-mass spectrum: *m/z* 734 (M^+). N.m.r. data (CD_3OD): ^1H (90 MHz), δ 2.6 (dd, 2 H, β-CH_2 Asn), 2.02, 2.0, 1.97, 1.91 (4 s, 12 H, 4 CH_3CO), 1.43 (s, 9 H, Me_3C), 1.39 (d, 3 H, *J* 6.7 Hz, CH_3), 1.19 (d, 3 H, *J* 6.2 Hz, CH_3).

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*N*-*tert*-butoxycarbonyl-L-leucyl-L-threonyl)-L-asparagine (**9a**); ν_{max}^{KBr} 1745 (C=O, ester) and 1655 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (22.63 MHz), δ 172.5–169.2 (8 s, 8 C=O), 155.3 (C=O, urethane), 78.2 [$(CH_3)_3C$ and C-1], 73.3 (C-3), 72.3 (C-5), 28.1 [$(CH_3)_3C$], 24.3 [$CH(CH_3)_2$], 22.9 and 22.5 [$CH(CH_3)_2$], 21.4 (CH_3CONH), 20.4 and 20.3 (CH_3COO), 18.9 [$CH(OH)CH_3$].

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*N*-benzoylglycyl-L-threonyl)-L-asparagine (**9b**); ν_{max}^{KBr} 1750 (C=O, ester) and 1660 cm^{-1} (Amide I). N.m.r. data [$(CD_3)_2SO$]: ^1H (90 MHz), δ 8.8 (s, 1 H, COOH), 8.7 (d, 1 H, *J* 10 Hz, NH), 8.0–7.3 (m, 8 H, 3 NH and Ph), 1.99, 1.96, 1.9, 1.74 (4 s, 12 H, 4 CH_3CO), 1.0 [d, 3 H, *J* 6.8 Hz, $CH(OH)CH_3$].

N-*Acylglycopentapeptide allyl esters* **10** *and* **11**. — To a solution of **8** or **9** (1 mmol) and **4** (1 mmol) in chloroform (20 mL) was added EEDQ (0.4 g, 1.6 mmol). The solution was stirred for 5–7 days and then concentrated *in vacuo*. The residue was purified by chromatography on silica gel (light petroleum–ethyl acetate → chloroform–methanol) and the product was recrystallized from methanol–ether. The yields, physical data, and elemental analysis are given in Table VI. Using this procedure, the following *N*-acylglycopentapeptides were prepared.

N^4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^2-(*tert*-butoxycarbonyl)-L-asparaginyl-L-alanyl-L-threonyl-L-isoleucyl-L-leucine allyl ester (**10**) was isolated by chromatography on silica gel (light petroleum–ethyl acetate → chloroform–methanol, 7:1), R_F 0.6 (chloroform–methanol, 7:1); ν_{max}^{KBr} 1750 (C=O, ester) and 1650 cm^{-1} (Amide I). F.a.b.-mass spectrum: *m/z* 1000 (M^+). N.m.r. data [$(CD_3)_2SO$]: ^1H (400 MHz), δ 8.57 (d, 1 H, $J_{NH,H-1}$ 9.6 Hz, NH Asn), 8.36 (d, 1 H, *J* 8 Hz, NH), 8.06 (d, 1 H, *J* 9 Hz, NH), 7.91 (d, 1 H, $J_{NH,H-2}$ 7.9 Hz, CH_3CONH),

7.85 (d, 1 H, *J* 7 Hz, NH Ala), 7.6 (d, 1 H, *J* 10 Hz, NH), 6.87 (d, 1 H, *J* 8 Hz, NH Asn), 5.87 (ddt, 1 H, C*H*=CH_2), 5.30 (dd, 1 H, J_{trans} 17.3, J_{gem} 1.5 Hz, CH=CH_{2trans}), 5.20 (dd, 1 H, J_{cis} 10.6, J_{gem} 1.5 Hz, CH=CH_{2cis}), 5.18 (dd, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 5.13 (dd, 1 H, $J_{3,4}$ 9.5, $J_{3,2}$ 7.3 Hz, H-3), 5.03 [d, 1 H, $J_{OH,\beta\text{-}CH}$ 5.6 Hz, CH(O*H*)CH_3], 4.81 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.54 (d, 2 H, *J* 5.6 Hz, CH_2CH=CH_2), 4.36 (dq, 1 H, α-CH Ala), 4.3–4.2 (m, 4 H, α-CH of Asn, Thr, Ile, and Leu), 4.18 (dd, 1 H, $J_{5,6}$ 3.9 Hz, H-6), 4.0–3.9 (m, 2 H, β-CH Thr and H-6'), 3.88–3.78 (m, 2 H, H-2,5), 2.57 (dd, 1 H, J_{gem} 15.1, J_{vic} 4.8 Hz, β-CH_a Asn), 2.42 (dd, 1 H, J_{vic} 6 Hz, β-CH_b Asn), 2.0, 1.97, and 1.91 (3 s, 9 H, 3 CH_3COO), 1.77 (s, 3 H, CH_3CONH), 1.77–1.70 (m, 1 H, β-CH Ile), 1.68–1.54 (m, 2 H, β-CH_a Leu and γ-CH Leu), 1.53–1.40 (m, 2 H, β-CH_b and α-CH_a Ile), 1.4 (s, 9 H, Me_3C), 1.2 (d, 3 H, *J* 6.1 Hz, β-CH_3 Ala), 1.14–1.02 (m, 1 H, γ-CH_b Ile), 1.01 [d, 3 H, *J* 6.2 Hz, CH(OH)CH_3], 0.9 (d, 3 H, *J* 6.1 Hz, δ-CH_{3a} Leu), 0.85–0.77 (m, 9 H, δ-CH_{3b} Leu, γ-CH_3 and δ-CH_3 Ile); ^{13}C (100.6 MHz), δ 172.1–169.3 (C=O), 155.1 (C=O urethane), 132.4 (*C*H=CH_2), 117.8 (CH=*C*H_2), 78.5 [$(CH_3)_3C$], 78.1 (C-1), 73.4 (C-3), 72.3 (C-5), 68.5 (C-4), 66.4 (β-CH Thr), 64.8 (*C*H_2CH=CH_2), 61.9 (C-6), 58.2 (α-CH Thr), 56.5 (α-CH Ile), 52.3 (C-2), 50.9 (α-CH Asn), 50.3 (α-CH Leu), 48.2 (α-CH Ala), 36.9 (β-CH Ile), 28.0 [$(CH_3)_3C$], 24.1 (β-CH_2 Leu), 23.9 (β-CH_2 Ile), 22.6 (CH_3 Leu), 22.5 (*C*H_3CONH), 21.0 (CH_3 Leu), 20.3 and 20.2 (*C*H_3*C*OO), 19.4 (CH_3 Thr), 15.2 (γ-CH_3 Ile), 11.1 (δ-CH_3 Ile).

*N*⁴-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*²-(*N*-*tert*-butoxycarbonyl-L-leucyl-L-threonyl)-L-asparaginyl-L-alanyl-L-threonine allyl ester (**11a**). This product was recrystallized from methanol–ether; R_F 0.36 (chloroform–methanol, 14:1); ν_{max}^{KBr} 1750 (C=O, ester) and 1650 cm^{-1} (Amide I). F.a.b.-mass spectrum: *m/z* 988 (M^+). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (20.15 MHz), δ 172.5–169.2 (C=O), 155.3 (C=O, urethane), 132.3 (*C*H=CH_2), 117.5 (CH=*C*H_2), 78.2 [$(CH_3)_3C$], 78.0 (C-1), 73.3 (C-3), 72.3 (C-5), 61.8 (C-6), 28.1 [$(CH_3)_3C$], 24.2 [$CH(CH_3)_2$], 23.0 and 22.6 [$CH(CH_3)_2$], 21.3 (*C*H_3CONH), 20.4 (*C*H_3COO), 20.0, 18.7, 18.0 (2 CH_3 Ala and CH_3 Thr).

*N*⁴-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*²-(*N*-benzoylglycyl-L-threonyl)-L-asparaginyl-L-isoleucyl-L-serine allyl ester (**11b**) was recrystallized from methanol–ether; ν_{max}^{KBr} 1745 (C=O, ester) and 1645 cm^{-1} (Amide I). F.a.b.-mass spectrum: *m/z* 964 (M^+). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (20.15 MHz), δ 171.2–169.1 (10 s, C=O), 166.7 (Ph-C=O), 134.0 (ipso-C, Ph), 132.4 (*C*H=CH_2), 131.4 (*p*-C, Ph), 128.3 (*m*-C, Ph), 127.3 (*o*-C, Ph), 117.6 (CH=*C*H_2), 78.2 (C-1), 73.3 (C-3), 72.3 (C-5), 24.1 (*C*H_2CH_3), 22.6 (*C*H_3CONH), 20.9, 20.5, 20.4 (*C*H_3COO), 19.2 [CH(OH)*C*H_3], 15.3 and 11.3 [$CH(CH_3)CH_2CH_3$].

N⁴-*(2-Acetamido-2-deoxy-β-D-glucopyranosyl)*-N²-*(*tert*-butoxycarbonyl)-L-asparaginyl-L-threonyl-L-serine amide* (**13**). — A solution of **5d** (0.6 g, 0.758 mmol) in saturated methanolic ammonia was stirred at room temperature for 3 days, then concentrated to dryness. The residue was recrystallized from 2-propanol–water, to give **13** (for the yield, physical data, and elemental analysis, see Table VII); ν_{max}^{KBr} 1645 (Amide I) and 1550 cm^{-1} (Amide II). F.a.b.-mass spectrum: *m/z* 622 and

623 (M^+). N.m.r. data (D_2O): ^1H (470 MHz), δ 5.07 (d, 1 H, $J_{1,2}$ 9.56 Hz, H-1), 4.56 (dd, 1 H, J 4.56 Hz, α-CH Asn), 4.45 (t, 1 H, J 5.06 Hz, α-CH Ser), 4.39 (d, 1 H, J 4.5 Hz, α-CH Thr), 4.32 (m, 1 H, β-CH Thr), 3.92–3.86 (m, 3 H, β-CH_2 Ser and H-6), 3.81 (dd, 1 H, $J_{2,3}$ 10.13 Hz, H-2), 3.75 (dd, 1 H, $J_{6',5}$ 5.06, $J_{6',6}$ 12.4 Hz, H-6'), 3.61 (dd, 1 H, $J_{3,4}$ 8.43 Hz, H-3), 3.53–3.45 (m, 2 H, H-4,5), 2.85 (dd, 1 H, J_{gem} 16.3, J_{vic} 5.6 Hz, β-CH_a Asn), 2.75–2.67 (m, 1 H, β-CH_b Asn), 2.02 (s, 3 H, CH_3CO), 1.44 (s, 9 H, Me_3C), 1.22 [d, 3 H, J 6.2 Hz, CH(OH)CH_3]: N.m.r. data (D_2O, CD_3OD): ^{13}C (22.63 MHz), δ 175.6–172.7 (C=O), 157.9 (C=O, urethane), 82.8 (C-1), 79.2 (C-3), 78.5 (C-5), 75.1 (C-4), 37.8 (β-CH_2 Asn), 28.5 [(CH_3)$_3$C], 24.7 (CH_3CO), 19.6 [CH(OH)CH_3].

N^4-*(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)*-N^2-*(*tert*-butoxycarbonyl)*-L-*asparaginyl*-L-*alanyl*-L-*threonyl*-L-*isoleucyl*-L-*leucine amide* (**14**). — Compound **10** (0.4 g, 0.4 mmol) was treated with methanolic ammonia (50 mL) as described above for compound **13**. The product was recrystallized from 2-propanol–water (the yield, physical data, and elemental analysis are given in Table VII); ν_{max}^{KBr} 1650 (Amide I) and 1550 cm^{-1} (Amide II). F.d.-mass spectrum: *m/z* 856 $(M + Na)^+$. N.m.r. data (D_2O): ^1H (470 MHz), δ 5.15 (d, 1 H, $J_{1,2}$ 9.56 Hz, H-1), 3.87 (dd, 1 H, $J_{6,5}$ 2, $J_{6,6'}$ 12.4 Hz, H-6), 3.81 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-2), 3.76 (dd, 1 H, $J_{6',5}$ 3.3 Hz, H-6'), 3.61 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 3.52–3.48 (m, 2 H, H-4,5), 2.82 (dd, 1 H, β-CH_a Asn), 2.7 (m, 1 H, β-CH_b Asn), 2.04 (s, 3 H, CH_3CO), 1.44 (s, 9 H, Me_3C), 1.2 [d, 3 H, J 6.2 Hz, CH(OH)CH_3], 1.19 (d, 3 H, J 5.1 Hz, CH_3 Ala), 0.95–0.85 (m, 12 H, 4 CH_3 Ile and Leu).

N^4-*(2-Acetamido-2-deoxy-β-*D*-glucopyranosyl)*-N^2-*(*N*-benzoylglycyl*-L-*threonyl)*-L-*asparaginyl*-L-*isoleucyl*-L-*serine amide* (**15**). — Compound **11b** (0.65 g, 0.67 mmol) was treated with methanolic ammonia as described above for **13**. The product was recrystallized from methanol–acetone (for the yield, physical data, and elemental analysis, see Table VII); ν_{max}^{KBr} 1640 (Amide I) and 1550 cm^{-1} (Amide II). F.a.b.-mass spectrum: *m/z* 797 (M^+). N.m.r. data (D_2O): ^1H (470 MHz), δ 7.87 (d, 2 H, $J_{o,m}$ 7.48 Hz, *o*-H Ph), 7.68 (t, 1 H, $J_{m,p}$ 7.54 Hz, *p*-H Ph), 7.58 (dd, 2 H, *m*-H Ph), 5.05 (d, 1 H, $J_{1,2}$ 9.75 Hz, H-1), 4.44 (t, 1 H, J 5.36 Hz, α-CH Ser), 4.4 (d, 1 H, J 4.31 Hz, α-CH Thr), 4.29 [m, 1 H, CH(OH)CH_3], 4.24 (s, 2 H, α-CH_2 Gly), 4.21 (d, 1 H, J 7.1 Hz, α-CH Ile), 3.91–3.87 (m, 3 H, β-CH_2 Ser and H-6), 3.83 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-2), 3.75 (dd, 1 H, $J_{6',6}$ 12.3, $J_{6',5}$ 4.5 Hz, H-6'), 3.6 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 3.52–3.48 (m, 2 H, H-4,5), 2.95 (dd, 1 H, J_{gem} 17.3, J_{vic} 6.6 Hz, β-CH_a Asn), 2.84 (dd, 1 H, J_{vic} 6.76 Hz, β-CH_b Asn), 2.01 (s, 3 H, CH_3CO), 1.93–1.87 (m, 1 H, β-CH Ile), 1.39 (dt, 1 H, γ-CH_a Ile), 1.26 (d, 3 H, J 6.4 Hz, Me Thr), 1.17 (dt, 1 H, γ-CH_b Ile), 0.89 (d, 3 H, J 7 Hz, CMe), 0.82 (t, 3 H, J 7.3 Hz, CH_2CH_3 Ile).

Cleavage of the Boc-group from N*-terminal protected asparaginylglyco-tripeptide or -pentapeptide* **13** *or* **14**. — A solution of **13** or **14** (0.1 mmol) in 3M HCl (5 mL) was stirred at room temperature for 1 h, then concentrated *in vacuo*. The residue was triturated several times with methanol and concentrated to dryness to

leave an amorphous residue in quantitative yield. Using this procedure, the following two glycopentapeptides were prepared.

*N*⁴-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-threonyl-L-serine amide hydrochloride (**16**). N.m.r. data (D_2O): ^1H (470 MHz), δ 5.1 (d, 1 H, $J_{1,2}$ 9.52 Hz, H-1), 4.47 (m, 2 H, α-CH Asn and α-CH Ser), 4.32 (d, 1 H, *J* 4.4 Hz, α-CH Thr), 3.77 (dd, 1 H, $J_{6',6}$ 11.8, $J_{6',5}$ 5.9 Hz, H-6'), 3.63 (dd, 1 H, $J_{3,2}$ 9.7, $J_{3,4}$ 9.1 Hz, H-3), 3.56–3.46 (m, 2 H, H-4,5), 3.07 and 3.0 (2 m, 2 H, β-CH_a and β-CH_b Asn), 2.03 (s, 3 H, CH_3CO), 1.26 (d, 3 H, *J* 6.6 Hz, CHCH_3).

*N*⁴-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-alanyl-L-threonyl-L-isoleucyl-L-leucine amide (**17**). N.m.r. data (D_2O): ^1H (470 MHz), δ 5.09 (d, 1 H, $J_{1,2}$ 9.56 Hz, H-1), 3.89 (dd, 1 H, $J_{6,6'}$ 2, $J_{6,5}$ 12.5 Hz, H-6), 3.82 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 3.77 (dd, 1 H, $J_{6',5}$ 4.5 Hz, H-6'), 3.63 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 3.56–3.47 (m, 2 H, H-4,5), 3.05 (dd, 1 H, J_{gem} 18, J_{vic} 4.7 Hz, β-CH_a Asn), 2.92 (dd, 1 H, J_{vic} 9.5 Hz, β-CH_b Asn), 2.02 (s, 3 H, CH_3CO), 1.44 [d, 3 H, *J* 6.7 Hz, CH(OH)CH_3], 1.21 (d, 3 H, *J* 6.8 Hz, CH_3 Ala), 0.95–0.87 (m, 12 H, 4 CH_3 Ile and Leu).

N⁴-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)*-N²-*(tert-butoxycarbonyl)*-L-*asparagine 3-(triethoxysilyl)propylamide* (**18**). — *(a) Using 3-(triethoxysilyl)propylamine.* A mixture of **2** (0.5 g, 0.9 mmol), EEDQ (0.26 g, 1 mmol), and 3-(triethoxysilyl)propylamine (0.2 g, 0.9 mmol) in chloroform (10 mL) was stirred at room temperature for 5 days and **18** was precipitated with light petroleum.

(b) Using 3-(triethoxysilyl)propyl isocyanate. To a solution of **2** (0.5 g, 0.9 mmol) in chloroform (10 mL) was added 3-(triethoxysilyl)propyl isocyanate (0.44 g, 1.78 mmol) dropwise. The mixture was stirred at room temperature for 2 days, and **18** was precipitated with light petroleum, collected, washed with light petroleum, and dried *in vacuo*. Compound **18** (0.6 g, 88%) had m.p. 190°, $[\alpha]_D^{22}$ +15° (*c* 1, chloroform); ν_{max}^{KBr} 1750 (C=O, ester) and 1660 cm^{-1} (Amide I). N.m.r. data ($CDCl_3$): ^1H (90 MHz), δ 3.8 (q, 6 H, *J* 7.1 Hz, 3 OCH_2CH$_3$), 2.08, 2.06, 2.03, and 2.01 (4 s, 12 H, 4 CH_3CO), 1.44 (s, 9 H, Me_3C), 1.22 (t, 9 H, *J* 7.1 Hz, 3 CH_2CH_3). N.m.r. data [$(CD_3)_2SO$]: ^{13}C (20.15 MHz), δ 171.1–169.3 (C=O), 155 (C=O urethane), 78.2 [$(CH_3)_3C$], 78.0 (C-1), 73.3 (C-3), 72.3 (C-5), 57.7 (3 OCH_2CH_3), 28.5 ($CH_2CH_2CH_2$), 28.1 [$(CH_3)_3C$], 22.6 (CH_3CONH), 20.4 and 20.3 (CH_3CO), 18.1 (3 OCH_2CH_3).

Anal. Calc. for $C_{32}H_{56}N_4O_{15}\cdot 2\,H_2O$: C, 47.99; H, 7.04; N, 6.99. Found: C, 47.90; H, 6.90; N, 7.64.

N⁴-*(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl*-N²-*(tert-butoxycarbonyl)*-L-*asparagine octadecylamide* (**20a**). — To a solution of **2** (0.25 g, 0.44 mmol) and ocatadecylamine (0.12 g, 0.44 mmol) in chloroform (5 mL) was added EEDQ (0.22 g, 0.89 mmol). The solution was stirred for 48 h at room temperature, then concentrated *in vacuo*. The residue was purified by chromatography on silica gel (light petroleum–ethyl acetate, 1:1 → chloroform–methanol, 7:1) (for the yield, physical data, and elemental analysis, see Table VIII); R_F 0.82

(chloroform–methanol, 7:1), ν_{max}^{KBr} 1750 (C=O, ester) and 1660 cm^{-1} (Amide I). N.m.r. data ($CDCl_3$): ^1H (60 MHz), δ 2.1–1.9 (4 s, 12 H, 4 CH_3CO), 1.4 (s, 9 H, Me_3C), 1.35–0.9 (m, 37 H, $C_{18}H_{37}$).

N^4-*(2-Acetamido-2-deoxy-β-D-glucopyranosyl)*-N^2-*(tert-butoxycarbonyl)*-L-*asparagine octadecylamide* (**20b**). — A solution of **20a** (0.35 g, 0.44 mmol) in chloroform (5 mL) and cold saturated methanolic ammonia (10 mL) was stirred for 24 h at room temperature, then filtered, and concentrated to dryness. The residue was purified by chromatography on silica gel (chloroform–methanol, 3:1) (for the yield, physical data, and elemental analysis, see Table VIII); R_F 0.15 (chloroform–methanol 7:1); ν_{max}^{KBr} 1690 (urethane) and 1660 cm^{-1} (Amide I). N.m.r. data ($CDCl_3$–CD_3OD, 1:1): ^1H (200 MHz), δ 4.91 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 2.61 (d, 2 H, J 5.9 Hz, β-CH_2 Asn), 1.45 (s, 9 H, Me_3C), 1.35–1.21 (m, 32 H, 16 CH_2), 0.88 (t, 3 H, J 6.3 Hz, CH_2CH_3).

N^4-*(2-Acetamido-2-deoxy-β-D-glucopyranosyl)*-L- *asparagine octadecylamide hydrochloride* (**20c**). — The amide **20b** (0.3 g, 0.44 mmol) was stirred with methanol (5 mL) and cold dichloromethane (50 mL), saturated with HCl, at room temperature. The reaction was monitored by t.l.c. (chloroform–methanol, 4:1). When the reaction was finished, the solution was concentrated *in vacuo,* the residue triturated with dichloromethane, and the solid collected by filtration (the yield, physical data, and elemental analysis are given in Table VIII); ν_{max}^{KBr} 1660 (Amide I) and 1550 cm^{-1} (Amide II). N.m.r. data (CD_3OD–$CDCl_3$–CF_3COOH, 10:10:1): ^1H (200 MHz), δ 5.0 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 2.02 (s, 3 H, CH_3CO), 1.3–1.2 (m, 32 H, 16 CH_2), 0.9 (t, 3 H, J 6.3 Hz, CH_2CH_3).

N^4-*(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)*-N^2-*(tert-butoxycarbonyl)*-L-*asparagine bis(octadecyl)amide* (**21a**). — A solution of **2** (0.6 g, 1.07 mmol), bis(octadecyl)amine (0.65 g, 1.19 mmol), and EEDQ (0.44 g, 1.8 mmol) in chloroform (10 mL) was stirred for 48 h at room temperature, then concentrated *in vacuo.* The residue was purified by chromatography on silica gel (light petroleum–ethyl acetate, 1:1) and the product was crystallized by trituration with ether (for the yield, physical data, and elemental analysis, see Table VIII); R_F 0.67 (ethyl acetate); ν_{max}^{KBr} 1750 (C=O, ester) and 1660 cm^{-1} (Amide I). N.m.r. data ($CDCl_3$): ^1H (90 MHz), δ 2.06, 2.04, 1.98, 1.8 (4 s, 12 H, 4 CH_3CO), 1.41 (s, 9 H, Me_3C), 1.35–1.2 (m, 74 H, 37 CH_2), 0.95–0.8 (m, 6 H, 2 CH_3).

N^4-*(2-Acetamido-2-deoxy-β-D-glucopyranosyl)*-N^2-*(tert-butoxycarbonyl)*-L-*asparagine bis(octadecyl)amide* (**21b**). — Compound **21a** (0.5 g, 0.46 mmol) was stirred in dichloromethane (20 mL) and methanolic ammonia (30 mL) for 24 h at room temperature. The solution was concentrated *in vacuo* and the residue was chromatographed on silica gel (chloroform–methanol, 7:1) (the yield, physical data, and elemental analysis are given in Table VIII); R_F 0.63 (chloroform–methanol, 7:1); ν_{max}^{KBr} 1690 (urethane) and 1650 cm^{-1} (Amide I). N.m.r. data ($CDCl_3$): ^1H (200 MHz), δ 4.99 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), 2.0 (s, 3 H, CH_3CO), 1.45 (s, 9 H, Me_3C), 1.3–1.2 (m, 74 H, 37 CH_2), 0.95–0.85 (m, 6 H, 2 CH_3).

N^4-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine bis(octadecyl)-amide (**21c**). — Compound **21b** (0.35 g, 0.4 mmol) was stirred with dichloromethane saturated with HCl (50 mL) for 3 h at room temperature. The crystals were collected and the filtrate was concentraed *in vacuo* (for the yield, physical data, and elemental analysis, see Table VIII); ν_{max}^{KBr} 1650 (Amide I) and 1550 cm^{-1} (Amide II). N.m.r. data (CD_3OD): ^1H (200 MHz), δ 5.0 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 2.7 (m, 2 H, β-CH_2 Asn), 1.96 (s, 3 H, CH_3CONH), 1.6–1.4 [broad, 4 H, $N(CH_2)_2$], 0.93–0.86 (m, 6 H, 2 CH_3).

Recording of the "pressure–surface diagram". — Compound **20c** (4.4 mg) was dissolved in 16:2:3 hexane–acetic acid–ethanol (20 mL) (solution *A*), and **21c** (5.5 mg) was dissolved in 8:2 ether–methanol (10 mL) (solution *B*). Portions (20 μL) of solutions *A* and *B* were added to a water surface in a film balance. The surface available for the molecules was continuously reduced and the resulting pressure against a stationary barrier was registered.

Preparation of the liposome solution. — A suspension of **21c** (10 mg) in water (3 mL) was treated with ultra-sound (Branson Ultrasonicator) for 8 min. The solution was filtered through a millipore gel-permeation filter, the first 3 mL eluted were discarded, and the following 3 mL of liposome-containing solution were collected. Small spherical particles were visualized with an electron microscope.

ACKNOWLEDGMENTS

This research was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We thank Professor Dr. H. Ringsdorf and Dr. R. Neumann, Universität Mainz, for their help in recording the surface–pressure diagrams, and Dr. M. Pook (Boehringer AG Ingelheim) for recording the 2D-n.m.r. spectra and for helpful discussions.

REFERENCES

1 G. ASHWELL, *Trends Biochem. Sci.*, 2 (1977) N-186.
2 H. KUNZ, *Angew. Chem.*, 99 (1987) 297–311; *Angew. Chem. Int. Ed. Engl.*, 26 (1987) 294–308.
3 H. KUNZ AND H. WALDMANN, *Angew. Chem.*, 96 (1984) 49–50; *Angew. Chem. Int. Ed. Engl.*, 23 (1984) 71–72.
4 H. WALDMANN AND H. KUNZ, *Justus Liebigs Ann. Chem.*, (1983) 1712–1725.
5 H. WALDMANN AND H. KUNZ, *Helv. Chim. Acta*, 68 (1985) 618–622.
6 (*a*) B. BELLEAU AND G. MALEK, *J. Am. Chem. Soc.*, 90 (1968) 1651–1652; (*b*) W. KÖNIG AND R. GEIGER, *Chem. Ber.*, 103 (1970) 788–798.
7 J. EMURA AND K. SCHMID, *Fed. Proc.*, 29 (1970) 1980.
8 L. MESTER, in K. LAKI (Ed.), *Fibrinogen*, Dekker, New York, 1980, p. 165.
9 E. M. TAGA, A. WAHEED, AND R. L. VAN ETTEN, *Biochemistry*, 23 (1984) 815.
10 L. HORNER AND M. VOGT, unpublished results.
11 K. K. UNGER AND B. ANSPACH, unpublished results.
12 J. FUHRHOP, *Nachr. Chem. Tech. Lab.*, 28 (1980) 792–797; L. GROS, H. RINGSDORF, AND H. SCHUPP, *Angew. Chem.*, 93 (1981) 311–332; *Angew. Chem. Int. Ed. Engl.*, 20 (1981) 305–325.
13 L. LANGMUIR, *J. Am. Chem. Soc.*, 39 (1917) 1848–1906.