General Methods for α- or β-O-Ser/Thr Glycosides and Glycopeptides. Solid-Phase Synthesis of O-Glycosyl Cyclic Enkephalin Analogues[1]

Robin Polt,* Lajos Szabó, Jennifer Treiberg,† Yushun Li, and Victor J. Hruby

Contribution from the Department of Chemistry, University of Arizona, Tucson, Arizona 85721.
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Abstract: O-Linked glycopeptides have been efficiently synthesized using the highly nucleophilic α-imino esters (O'Donnell's Schiff bases) derived from L-serine (**3a–c**), L-threonine (**4a,b**), and a dipeptide ester (**5**). General methodology has been developed which can provide β-glycosides of β-hydroxy-α-amino acid derivatives **6–16** in excellent yield (63–94%) and excellent selectivity (>20:1) using Hanessian's modification or Helferich's modification of the Koenigs–Knorr reaction. Likewise, selective α-glycosylation has been achieved using the in situ anomerization methodology of Lemieux (**28, 30**). The increased nucleophilicity of the serine/threonine hydroxyl has been shown to be due to intramolecular hydrogen bonding to the N=CPh$_2$ moiety. Deprotection of the intermediate Schiff bases has been demonstrated, and the glycosides have been incorporated into fully deprotected O-linked glycopeptides in high yield using either solution-phase peptide methods or solid-phase Fmoc-based technology. The potent glycosylenkephalin analogue **38** has been prepared using the solid-phase methodology.

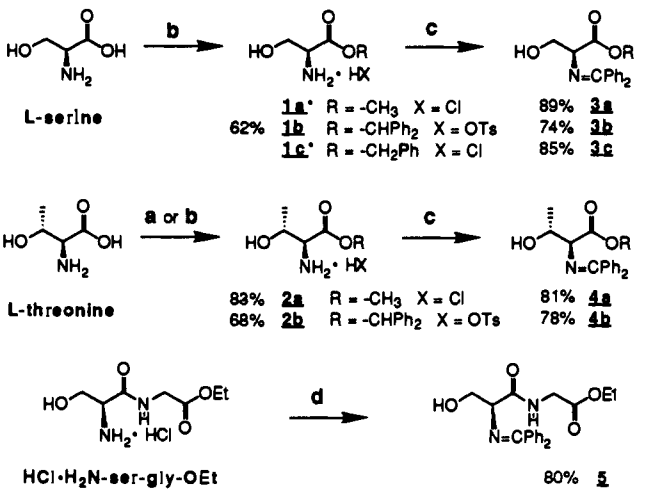
Introduction. In naturally occurring glycoproteins and in the glycopeptide hormones, the carbohydrate moieties play key roles in the intercellular and intracellular transport of the gene products (exit passport hypothesis),[2] as well as extend the biological half-life of the active peptides in vivo (proteolytic protection). Additional roles supported by experimental evidence include the alteration of peptide backbone conformation (protein folding),[3] control of membrane permeability, and molecular recognition (the concept of carbohydrate "antennae").[4] These concepts have been summarized and amplified by Montreuil[5] and have given birth to the field of "glycobiology".[6] The chemical synthesis of glycopeptides[7] provides an important tool for the study of glycopeptide hormones, glycoproteins, and other complex carbohydrate structures found at the cell surface and in the glycocalyx.

Complex glycosides attached to exterior cell surfaces (N-linked glycoproteins, O-linked glycoproteins, and glycolipids) are involved in the regulation of cell metabolism, host–pathogen interactions, tumor cell metastasis, cell–cell recognition, cell adhesion, and cell development.[5] In order to fully define the roles complex carbohydrates play in these processes, and to thus understand "glycobiology" in its broadest sense, the synthesis of glycopeptides, glycolipids, and their structural analogues is required just as the chemical synthesis of DNA was required to understand molecular biology. Our focus in this article is on the synthesis of O-linked glycopeptides, which are not as well understood as their N-linked counterparts.

Abnormalities in O-linked glycopeptides are implicated in numerous disease states. Abnormal posttranslational modification of the τ protein has been implicated in the formation of neurofibrillary tangles of Alzheimer's disease.[8] The antigenic T-epitopes and T$_N$-epitopes of cell-surface glycopeptides have long been associated with cancer and have been used as tumor cell markers.[9] O-Glycosylated peptide fragments of these two proteins have been synthesized. Insulin-like growth factor (IGF-1),[10] the oncofetal fibronectin Val-Thr-His-Pro-Gly-Tyr fragment,[11] *O*-glycosylsomatostatin analogues,[12] *O*-glycosyltuftsin analogues,[13] *O*-glycosylmorphiceptin analogues,[14] glycophorin fragments,[15] and mucin fragments[16] have been synthesized for various biological studies.

The synthesis of O-linked glycopeptides is complicated by the acid-lability of glycosides in general and the base-sensitivity (retro-Michael reaction) of the *O*-serinyl and *O*-threonyl glycosides in particular.[17] Although Boc has been used for N-terminus protection,[12a] use of the Fmoc-based peptide coupling strategies (solution-[9b,11] or solid-phase[10] methodology) or Cbz-based strategies[13–15] avoids acidic conditions for deprotection of the N-termini and appears to be superior. Glycosylation of intact, resin-bound peptides has been attempted,[8] but does not appear to be a generally applicable approach. Acid-labile resin linkers[14,16,18] permit cleavage

Scheme I[a]



[a] (a) MeOH/$SOCl_2$/0 °C. (b) (1) TsOH; (2) $Ph_2C{=}N_2$/DMF/60 °C. (c) $Ph_2C{=}NH$*/CH_2Cl_2/room temperature. (d) $Ph_2C{=}NH$/CH_3CN/room temperature. *Commercial material (Aldrich, Sigma Co.).

† NSF-REU summer research participant.

(1) For a preliminary account, see: Szabó, L.; Li, Y.; Polt, R. *Tetrahedron Lett.* **1991**, *32*, 585–8.
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(4) Winterburn, P. J.; Phelps, C. F. *Nature (London)* **1972**, *236*, 147–151.
(5) Montreuil, J. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 157–223.
(6) Perhaps it is a credit to the proteoglycan/glycoprotein researchers that this area of active research is now much too important to be referred to by the term "carbohydrate chemistry", as in the conversion of "nucleic acid chemistry" to "molecular biology". See: Mohr, H. In *Reductionism and Systems Theory in the Life Sciences*; Hoynigen-Huene, P., Wuketits, F. M., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1989; pp 137–159.

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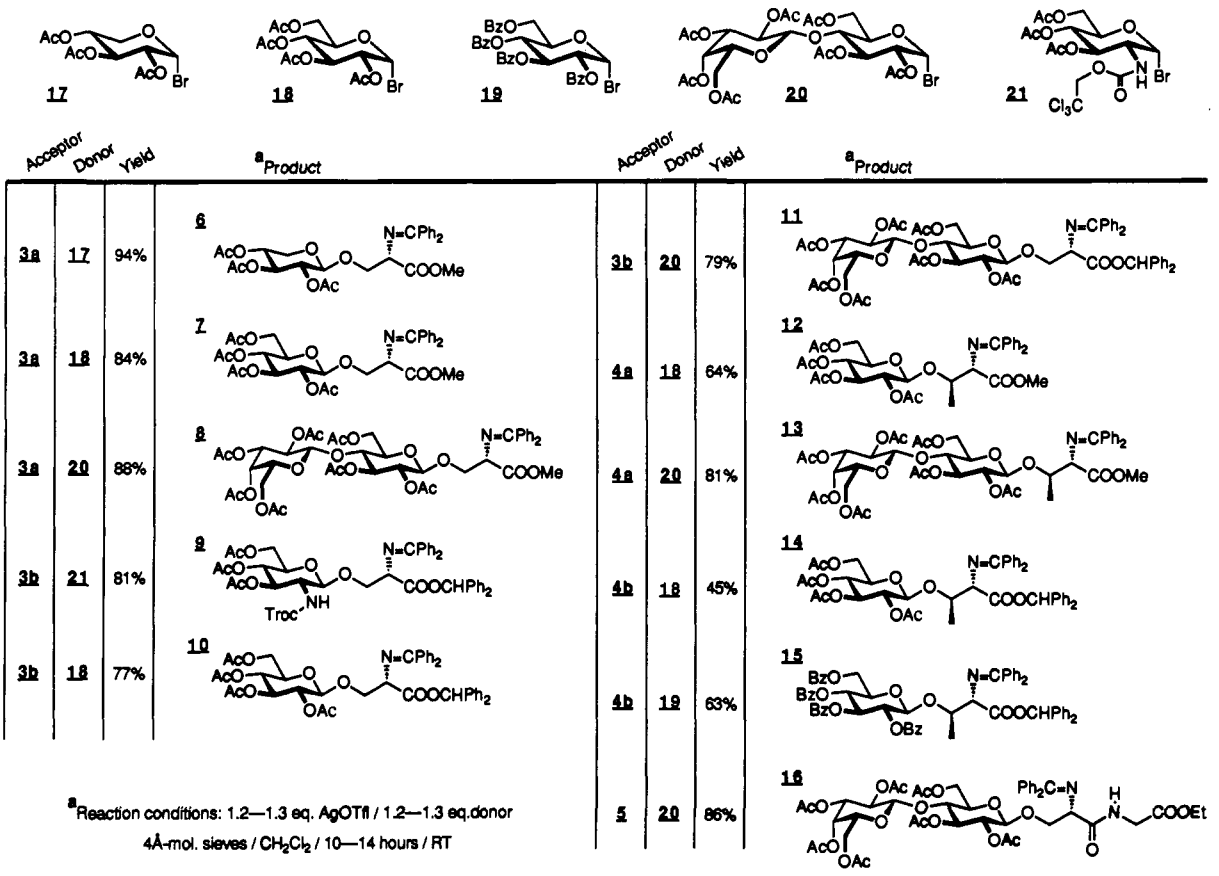
Scheme II



of the O-linked glycopeptides from the solid-phase support without exposure of the glycoside-bearing residues to strong acids. Thus, many of the problems encountered by earlier workers in the field have now been alleviated by modern solid-phase peptide methodology based on the Fmoc protecting group.[19]

The stereoselective synthesis of protected amino acid glycosides remains challenging. Glycosylation of N-acylated β-amino alcohols such as Fmoc-protected or Cbz-protected serine and threonine derivatives, as well as the structurally related ceramides and protected sphingosines,[20] is not straightforward. Problems encountered by the pioneers[17,21] in this area include low yields and poor α/β selectivity. We attribute these problems to the decreased nucleophilicity of the glycosyl acceptor due to an unfavorable hydrogen-bonding pattern. Conversely, we reasoned that if a favorable hydrogen-bonding pattern was generated, then the nucleophilicity of the neighboring hydroxyl would be increased (see Figure 1). Preliminary experiments[1] have confirmed this hypothesis.[20,22] We would like to report the use of this concept in the development of methods for the synthesis of either α- or β-O-linked glycopeptides and its application to the synthesis of an *O*-glycosyl analogue of DPDPE,[23] a potent δ-opioid receptor selective agonist.[24]

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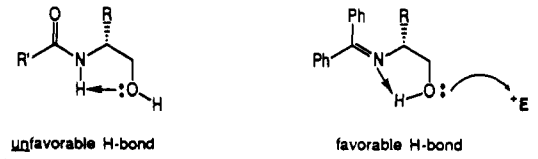
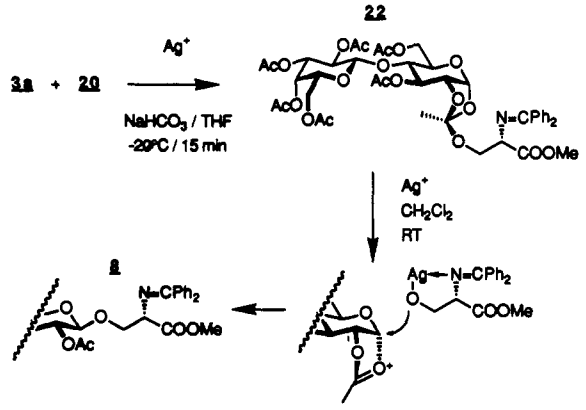


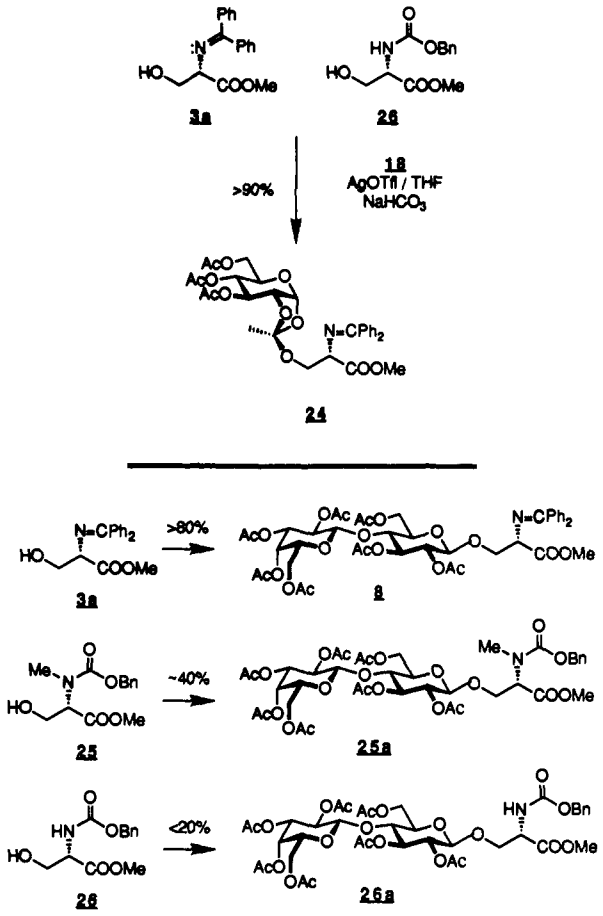
Figure 1.

Scheme III



Nucleophilic Serine and Threonine Schiff Bases. Synthesis of β-Glycosides via the Hanessian Modification of the Koenigs–Knorr Procedure. The required serine and threonine ester Schiff bases **3a–c**, **4a,b**, and **5** were prepared from diphenylketimine (benzophenone imine) and the appropriate α-amino ester hydrochloride salts or tosylate salts using the standard methodology published by O'Donnell and Polt[25,27] (see Scheme I). The benzhydryl ester was chosen for carboxyl protection because of its easy hydrogenolysis (vide infra) and because its steric bulk affords greater protection than benzyl or allyl. All of the compounds in Scheme I were crystalline. Solution ^1H and ^{13}C NMR studies indicated that each β-hydroxy Schiff base exists in a tautomeric equilibrium

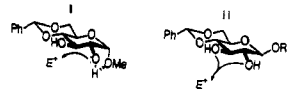
Scheme IV



with the cyclic oxazolidine form. X-ray analysis of **4a** showed that it had crystallized as the oxazolidine structure.[26] With the exception of the extremely unhindered electrophile PhN=C=O,[27] we have always observed reaction at the oxygen[28] of the open-chain β-hydroxy imine tautomer with a variety of electrophiles—including the glycosyl donors which form the subject matter of this article.

Glycosylation of the β-hydroxy Schiff bases with "participating" glycosyl donors was straightforward and gave the expected 1,2-trans products (β-glycosides). Although Helferich's glycosylation method[29] ($Hg(CN)_2/PhCH_3$–CH_3NO_2) worked well, Hanessian's modification of the Koenigs–Knorr reaction[30] was superior. Thus, the Schiff bases **3–5** were treated with various acyl-protected glycosyl bromides **17–21** in CH_2Cl_2 at room temperature for several hours with AgOTfl as a promoter to provide the desired β-glycosides **6–16** in good to excellent yields (Scheme II). The glycosides were easily purified on SiO_2 by flash chromatography,[31] and the corresponding 1,2-cis products (α-glycosides) could not be detected by either 250-MHz ^1H NMR or by thin-layer chromatography. There is some dimunition in yield as the steric bulk of the glycosyl acceptor is increased (Ser → Thr and COOMe → $COOCHPh_2$); thus **4b** is significantly less reactive than **3a** (cf. 45% yield of **14** vs 84% yield of **7**). Use of a more reactive glycosyl

(22) Enhanced nucleophilicity of hydroxyl groups via intramolecular H-bonding has been offered as an explanation for selective O-tosylation: (a) Denis, J. N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1990**, *55*, 1957. The α-methyl glucopyranoside i is selectively glycosylated at the 2-hydroxyl (favorable H-bond to the anomeric oxygen), whereas the corresponding β-glycosides ii show no such selectivity: (b) Bochkov, A. F.; Zaikov, G. E. *The Chemistry of the O-Glycosidic Bond: Formation & Cleavage*; Pergamon Press, Oxford; 1979; p 100 and references therein.



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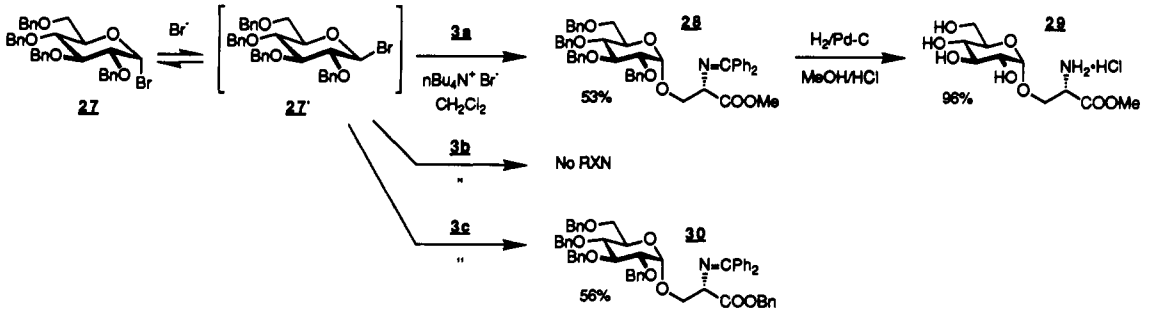
Scheme V



donor, such as perbenzoate **19**, partially offsets this diminished reactivity (cf. 63% yield of **15**). The dipeptide Schiff base **5** is quite reactive, in spite of the potentially "unfavorable" H-bond between the amide NH and the hydroxyl which has been observed in small threonine- and serine-containing peptides.[33]

Small amounts of the corresponding orthoesters were isolated from the reaction mixtures, and when the glycosylation reactions were run in THF with solid $NaHCO_3$ as a buffer, orthoesters (**12a**, **22–24**) were isolated in excellent yield after 5–10 min at −20 °C. When the crystalline orthoester **22** was treated with AgOTfl, $HgBr_2$, or $Me_3SiOTfl$ in CH_2Cl_2, glycoside **8** was obtained in high yield (Scheme III), suggesting that the orthoesters are indeed reaction intermediates in the Koenigs–Knorr reaction as independently proposed by Garegg and by Schroeder (inter alia).[32]

FT-IR measurements of **3a** in dilute $CHCl_3$ solution show a sharp O–H stretching peak which is shifted ($\Delta\nu_{OH}$) from the expected value by approximately 340 cm^{-1} to lower wavenumbers. This is consistent with a strong intramolecular hydrogen bond.[34] Several experiments were performed to determine whether the benzophenone-protected α-imino esters (favorable H-bond; cf. **3a**) are in reality more nucleophilic than the Cbz-protected α-amido esters (unfavorable H-bond; cf. **26**) and to examine the hydrogen-bonding hypothesis (Scheme IV). In competition experiments, 2 equiv each of glycosyl acceptors **3a** and **26** were allowed to react with 1 equiv of acetobromoglucose for 15 min at 0 °C in THF in the presence of 1 equiv of AgOTfl and excess solid $NaHCO_3$. Orthoester **24** was the only coupling product observed in crude reaction mixtures by 250-MHz ^1H NMR and was isolated in over 90% yield. One explanation of the increased reactivity of **3a** is that the intramolecular hydrogen bond in **3a** increases the electron density on the hydroxyl group, thereby increasing the nucleophilicity of the oxygen, whereas the hydrogen bonding in **26** serves to remove electron density from the hydroxyl, thereby decreasing the nucleophilicity of the oxygen.

A plausible alternative explanation is that the bidentate β-hydroxy imine **3a** may coordinate to Ag^+ (or Hg^{2+} in the case of the Helferich reaction[29]), causing a proximity effect[35] which favors the reaction of **3a** with the glycosyl donor. Both of these arguments are consistent with Garegg's suggestion[32c] that increasing the basicity of the glycosyl acceptor increases the rate in the Koenigs–Knorr reaction. Since the bidentate Schiff bases are much more basic than a typical primary alcohol, the nucleophilicity of the glycosyl acceptor is increased, as well as the basicity of the intermediate orthoester which must undergo proton or metal-catalyzed rearrangement to the β-glycoside product.

In three separate experiments (Scheme IV), the reactivity of **3a**, **25**, and **26** with acetobromoglucose **18** and AgOTfl was compared using Hanessian's method.[30] The result of each experiment is in agreement with the hydrogen-bonding hypothesis, that is, **3a** (favorable H-bonding) provided a high yield (>80%) of glycoside, **26** (unfavorable H-bonding) provided a low yield (<20%), and **25** (no H-bonding) provided an intermediate yield of the glycoside (~40%). Thus, while we cannot rule out other effects (i.e., chelation of the Ag^+ promoter and/or basicity of the hydroxyl), hydrogen bonding of the glycosyl acceptor certainly plays an important role in glycosylation reaction rates,[20] as well as in other related reactions between electrophiles and alcohols.[22]

All of the ^1H NMR chemical shift assignments (δ) and most of the coupling constants (hertz) for the β-glycoside products were provided by COSY and are listed in Table I. The ^{13}C NMR chemical shift assignments (δ) are listed in Table II. All of our ^{13}C NMR data were consistent with published data on glycosides,[51] which was very helpful in making the correct assignments. It should be noted that the *N*-methyl amino acid derivative **25a** shows two rotamer populations in the ^{13}C NMR spectrum, as might be expected for a tertiary amide structure.

Synthesis of α-Linked Glycosides via Lemieux's in Situ Anomerization Method. Lemieux's in situ anomerization method[36] (nBu$_4$N$^+$Br$^-$/iPr$_2$NEt/CH_2Cl_2) was applied to **3a** using the perbenzyl bromoglucose **27**[36] as a glycosyl donor (Scheme V). Presumably, the equatorial anomer **27′** is the reactive species. While the relatively unhindered methyl ester **3a** provided a 53% yield of α-glycoside **28**, the more sterically hindered benzhydryl ester **3b** completely failed to react. This is due to the increased steric demand of the S_N2-like transition state of the Lemieux conditions, coupled with the 1,3-diaxial interactions generated by the approach of the glycosyl acceptor. The Koenigs–Knorr transition state shows less sensitivity to steric hindrance because of the equatorial approach of the nucleophile and the increased S_N1 character. Attempts to force the Lemieux reaction to completion by adding DMF or warming the reaction resulted in elimination of HBr from the glycosyl donor to form the benzylated glucal. The less hindered benzyl ester **3c** reacted with **27** under identical conditions to provide the α-glycoside **30** in 56% yield. In both cases, only small amounts (~20:1) of the corresponding β-products could be observed in the ^1H NMR of the crude reaction mixtures of glycosides **28** and **30**.

Hydroxyl, Amino, and Carboxyl Deprotection and Solution-Phase Coupling of the Amino Acid Glycosides. The synthesis of O-linked glycopeptides requires protecting groups which can be removed under neutral, mildly acidic, or mildly basic conditions.[17] While the OH, NH_2, and COOH protecting groups used in this study cannot be removed in a completely orthogonal manner,[37] the sterically hindered $Ph_2C{=}N$ moiety does provide several convenient routes for deprotection (Scheme VI). Treatment of Schiff base glycosides with mild aqueous acid (e.g., 0.1–1.0 N aqueous HCl/Et_2O or THF or 5% citric acid) at 0 °C or room temperature for several hours provided the amino ester glycosides as free bases or as the HCl salts.[1,25] Similarly, acid-catalyzed hydrolysis (1.05–1.20 equiv of CF_3COOH) of the imine moiety of glycosides **7** and **10** also facilitated the selective removal of the amino protecting group in moist (1–2% water) CH_2Cl_2 or THF gave **31** and **33**, as did 2 equiv of $TsOH{\cdot}H_2O$ in THF. As free bases, the more hindered benzhydryl amino esters[38] (e.g., **33**) were

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Table I. ^{1}H NMR Data for Glycosides[a]

	8	11	13		8	11	13
			Chemical Shifts (ppm)				
H-1	4.58	4.53	4.64	H-4′	5.32	5.31	5.31
H-2	4.85	4.83	4.83	H-5′	3.80	3.76	3.76
H-3	5.16	5.10	5.14	H-6′a	4.07	4.06	4.10
H-4	3.73	3.76	3.72	H-6′b	4.07	4.06	4.10
H-5	3.56	3.46	3.41	α-H	4.34	4.36	4.08
H-6a	4.36	4.33	4.06	β-H	4.14	4.20	4.36
H-6b	4.03	4.06	3.92	β′-H	4.00	4.06	
H-1′	4.39	4.34	4.33	OCH_3	3.69		3.66
H-2′	5.09	5.06	5.06	CH_3			1.14
H-3′	4.91	4.89	4.89	$CHPh_2$		6.84	
			Coupling Constants ($J_{H,H}$, Hz)				
1,2	7.6	7.5	7.6	2′,3′	10.6	8.5	10.2
2,3	9.1	8.9	9.1	3′,4′	3.4	3.4	2.5
3,4	9.1	8.8	9.1	4′,5′	3.4	3.6	2.5
4,5	8.9	8.4	9.0	5′,6′	6.7	nd	7.0
5,6a	2.5	2.0	2.2	α,β	5.5	7.7	6.6
5,6b	nd	nd	4.3	α,β′	4.5	7.7	
6a,6b	nd	11.1	12.0	β,β′	10.1	nd	
1′,2′	7.8	7.7	7.8	β,CH_3			6.3

	6	7	9	10	12	14	15	31	33	34[b]
				Chemical Shifts (ppm)						
H-1	4.57	4.62	4.70	4.57	4.64	4.64	5.00	4.58	4.57	4.76
H-2	4.87	4.95	3.53	4.93	4.92	4.91	5.47	4.97	4.94	4.91
H-3	5.13	5.19	5.10	5.13	5.16	5.13	5.82	5.19	5.17	5.05
H-4	4.89	5.05	5.01	5.03	5.03	5.01	5.58	5.07	5.06	5.26
H-5	4.05	3.63	3.59	3.54	3.49	3.39	3.82	3.69	3.61	3.89
H-5′	3.31									
H-6		4.21	4.22	4.19	4.06	3.98	4.11	4.25	4.23	4.29
H-6′		4.03	4.02	4.98	3.71	3.55	4.02	4.14	4.09	4.11
α-H	4.36	4.35	4.43	4.43	4.12	4.20	4.21	3.62	3.67	3.86
β-H	4.10	4.14	4.13	4.20	4.35	4.37	4.47	4.09	4.28	4.18
β′-H	3.99	4.09	4.13	4.13				3.80	3.87	3.96
OCH_3	3.70	3.69			3.68			3.73		
CH_3					1.15	1.08	0.97			
$CHPh_2$			6.90	6.86		6.85	6.80		6.87	
CH_2			4.72							
CH_2′			4.23							
				Coupling Constants ($J_{H,H}$, Hz)						
1,2	6.4	7.9	8.4	7.8	7.9	7.9	7.9	7.9	7.9	7.9
2,3	8.3	9.5	9.4	9.2	9.5	9.4	9.8	9.5	9.3	9.6
3,4	8.3	9.4	9.4	9.3	9.5	9.5	9.7	9.5	9.4	9.5
4,5	4.6	9.6	9.5	9.5	9.5	9.6	9.8	10.0	9.4	9.7
4,5′	8.5									
5,5′	12.2									
5,6		4.3	4.3	4.5	4.0	3.7	3.0	4.8	4.4	4.3
5,6′		2.5	2.0	2.3	2.3	2.3	3.4	2.5	2.4	2.4
6,6′		12.2	12.1	12.3	12.3	12.3	12.2	12.3	12.5	12.8
α,β	6.5	6.0	5.9	6.1	7.0	7.4	7.7	5.0	4.3	2.9
α,β′	5.5	6.0	5.9	5.7	7.0			4.2	3.7	4.1
β,β′	10.9	6.4	5.6	10.2				9.9	10.0	11.2
β,CH_3					6.3	6.3	6.7			
CH_2,CH_2′			12.1							

[a] All NMR data is for solution in $CDCl_3$ unless otherwise indicated. [b] Measured in CD_3OD_3.

more stable than their methyl ester counterparts which formed diketopiperazines under basic conditions. Hydrogenolysis of **16** in the presence of di-*tert*-butyl pyrocarbonate (Boc_2O) and solid $NaHCO_3$ provided the Boc-protected amino ester **32**. Simultaneous hydrogenolysis (1 atm of H_2/5–10% Pd-C/MeOH) of the $N{=}CPh_2$ and $OCHPh_2$ groups from **10** provided the glycosyl amino acid **34** in excellent yield. Similarly, the benzyl groups used for protection of OH in the Lemieux procedure could be removed by hydrogenolysis simultaneously with the Schiff base (cf. Scheme V, **28** → **29**). Deacylation of the blocked sugar moieties could not be accomplished using Zemplèn conditions[39] (catalytic NaOMe/MeOH) without the first removing the Schiff base, which has an acidifying effect on the α-hydrogen of the amino acid residue,[40] thus promoting retro-Michael addition of the glycoside.

Solution-phase amino acid coupling of the protected amino ester glycosides proceeds without difficulty (Scheme VI). Thus, protected dipeptide glycoside **35** was synthesized in 84% yield from **34** and Boc-L-Phe using classical HOBT/DCC methodology.[41] Once the amino terminus has been acylated, either with Boc, Z (Cbz), or another amino acid residue, one can remove the acetate groups which protect the hydroxyls using the classical Zemplèn procedure[39] with no diketopiperazine formation (cf. Scheme VI).

Solid-Phase Coupling, Deprotection, and Cleavage of O-Linked Glycopeptides. Synthesis of a Potent *O*-Glycosyl DPDPE Ana-

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Table II. ^{13}C NMR Shifts of Glycosides[a]

no.	6	7	8	9	10	11	12	13	14	15	16	25a	26a	28	29[b]	30	31	32	34[c]
C-1	100.81	101.05	100.20	100.95	100.85	100.29	99.38	98.51	100.06	100.89	99.79	99.61, 99.17	100.76	97.20	101.04	97.38	100.74	100.96	101.90
C-2	70.49	71.07[d]	70.67[d]	55.69	70.91	70.91[d]	70.59	70.61[d]	71.25[d]	71.48	70.77[d]	70.44[d]	70.85[d]	79.79	73.17	79.98	70.89	70.87[d]	71.88
C-3	71.27	72.79	71.30	71.37	72.70	71.57	72.62	71.80	72.90	73.07	71.45	71.11	71.23	81.82	74.64[d]	82.04	72.45	71.44	73.20
C-4	68.79	68.26	75.89	68.28	68.02	76.16	67.83	75.74	68.00	69.57	75.94	75.91, 75.84	75.94	77.32	71.46	77.53	68.04	76.09	68.92
C-5	61.75	71.70[d]	72.23[e]	72.15	71.52	72.37	71.41	72.17[e]	71.69[d]	72.48[d]	72.32	72.43	72.44[e]	70.20	74.43[d]	70.37	71.59	72.49	72.37
C-6		61.75	61.75	61.64	61.55	62.04	61.24	61.57	61.35	62.55	61.81	61.64, 61.47	61.70	68.61	62.57	68.72	61.60	61.82	62.17
C-1′			100.79			101.07		100.75			100.90	100.81	100.99					101.43	
C-2′			68.78			68.99		68.68			68.83	68.85	68.97					68.94	
C-3′			70.32[d]			70.63[d]		70.21[d]			70.41[d]	70.23[d]	70.58[d]					70.60[d]	
C-4′			66.38			66.57		66.30			66.43	66.41	66.50					66.53	
C-5′			72.64[e]			73.04		72.76[e]			72.88	72.43	72.85[e]					72.88	
C-6′			60.58			60.78		60.50			60.64	60.63	60.73					60.73	
C=N	172.10	172.14	171.81	172.30	172.19	172.23	171.54	171.33	171.70	171.82	171.51			172.21		172.52			
α-C	65.29	65.29	64.96	65.62	65.56	65.54	71.41	70.67	71.84[d]	72.25[d]	66.14	58.49, 58.26	52.68	65.29	54.44	65.58	54.42	53.21	58.75
β-C		65.29	70.67	70.06	70.57	70.27	69.95	77.09	76.29	77.86	78.39	71.36	66.49	69.23	66.29	68.31	71.59	70.38	68.19
CH_3								16.66	16.42	17.14	17.32							14.06	
OCH_3		52.14	52.14	52.94				51.77	51.74			52.11		52.68	52.15	53.93		52.07	
$HC(Ph)_2$																			
CH_2CCl_3				77.05	77.23	77.42			77.25	77.27									
NCH_3				73.84								32.39, 32.04							

[a] Published NMR data was useful in making correct assignments;[51] measured in $CDCl_3$ unless otherwise indicated. [b] Measured in CD_3OD. [c] For a solution in CD_3OD_3. [d,e] Assignments in the same column may be reversed.

logue. Workers attempting solid-phase glycopeptide synthesis have been hampered by three intrinsic problems: (1) deprotection of the growing peptide chain; (2) removal of the hydroxyl protection on the carbohydrate moiety; and (3) cleavage of the completed glycopeptide from the support resin. These problems are especially acute when synthesizing O-linked glycopeptides which are base-labile at the serine or threonine residue, in addition to the normal acid-sensitivity shown by the glycosidic acetals.

The Fmoc-based peptide methodology is ideally suited to glycopeptide synthesis[42] since the deprotection step (piperidine/DMF) is compatible with most hydroxyl protecting groups commonly used in polysaccharide constructions. Stepwise or simultaneous removal of the $OCHPh_2$ and $Ph_2C{=}N$ groups from **10** as described previously, followed by reprotection of the amino group with Fmoc-Cl or Fmoc-OSu[19] (Scheme VI), provided protected glycosyl amino acid **36** suitable for solid-phase coupling using Castro's BOP reagent.[43] The coupling strategy is depicted in Scheme VII. Because Fmoc-protected serine glycosides (cf. **36**) are much more sterically encumbered than the typical proteogenic amino acids normally encountered in solid-phase peptide synthesis, it was not surprising that coupling of the carbohydrate-bearing residue was slower (~4 h) than usual. The following coupling to the terminal glucosyl-bearing serine was normal, as were subsequent couplings. One point is worth mentioning here: when **36** was coupled directly to the resin, extended reaction times (>24 h) were required, and acylation was never complete. Apparently, some of the acylation sites on the resin were not available to the bulkier glycosyl Fmoc-amino acid. Acetamidomethyl[44] groups were used to protect the sulfhydryl groups of the Fmoc-cysteine residues. The *tert*-butyl group was used for protection of the tyrosine hydroxyl, but was not required for coupling. Deprotection of the amino termini with piperidine in DMF was accomplished in the normal fashion after each coupling step.[19]

Removal of acetate groups from the carbohydrate portion of O-linked glycopeptides is still a particularly vexing problem since the usual basic conditions used for removal[39] lead to loss of the carbohydrate.[17] We have utilized a procedure developed by Kunz[45] that relies on aqueous hydrazine in MeOH to remove the acetates nucleophilically. Since the glycopeptide was still bound to the support at this stage, separation of the soluble acethydrazide byproduct was accomplished by simply washing the resin with CH_2Cl_2.

Due to the presence of the acid-labile *O*-glycoside, an extremely acid-sensitive trialkoxybenzhydryl-type linkage was required for efficient cleavage of the deprotected glycopeptides from the polymer support. Because we required a carboxamide C-terminus on the enkephalin analogues, we chose the benzhydrylamine-functionalized polystyrene developed by Rink[18] as a support. Thus, cleavage with TFA/CH_2Cl_2 provided 200 mg of the desired primary amide glycopeptide **37** with concomitant cleavage of the *tert*-butyl group on tyrosine. After cleavage from the polymer support, the sulfhydryl-protecting acetamidomethyl groups were removed with Hg^{2+},[44] and the disulfide bond was formed with $K_3Fe(CN)_6$ in dilute aqueous solution[46] to provide the biologically active[47] enkephalin analogue **38**. The overall yield, based on the resin, was 28%. FAB-MS and ^1H NMR indicated that the β-glucoside had been retained and that no detectable anomerization had occurred during deprotection and cleavage of the peptide from support. A series of glycosylated enkephalin analogues has been

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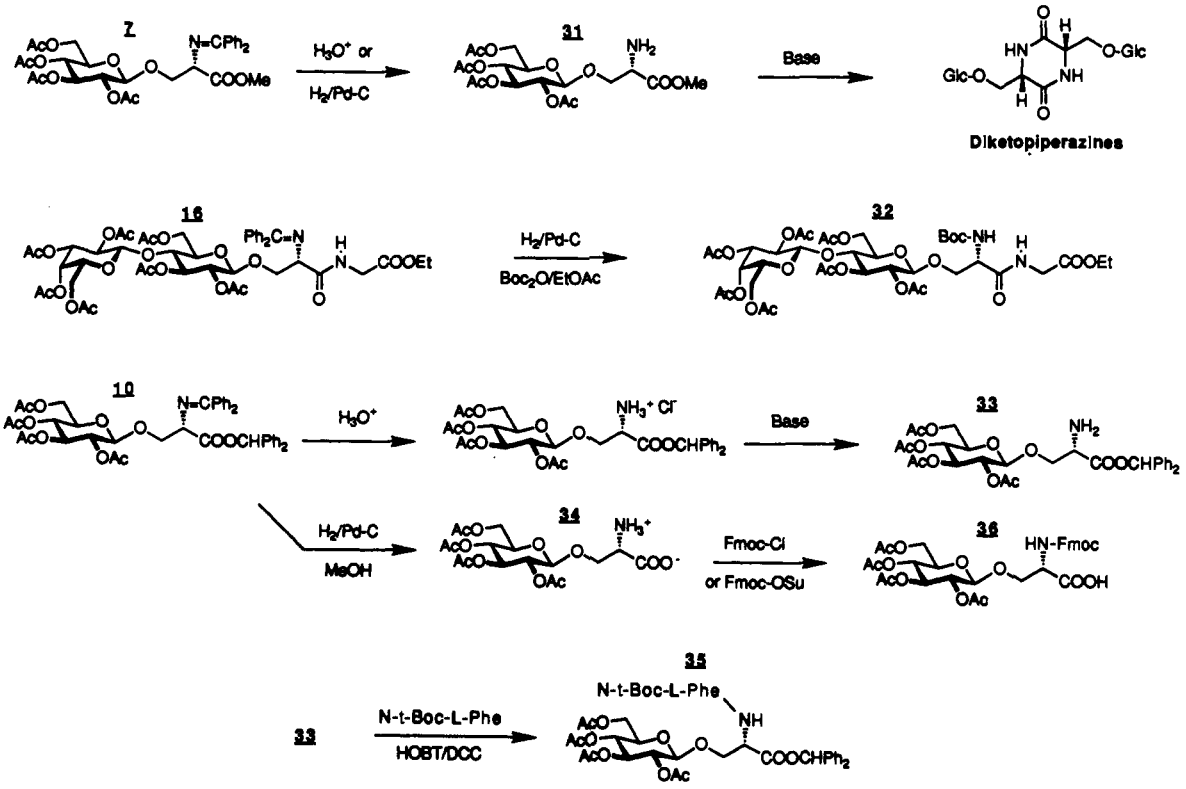
Scheme VI



prepared using this methodology, and the studies along with biological activities will be reported separately.[47]

Conclusions. An intramolecular hydrogen bond (C=N: → H—O:) has been used to enhance the nucleophilicity of serine and threonine hydroxyl groups. Benzophenone Schiff bases of α-amino esters (O'Donnell's Schiff bases[25]) have been shown to be useful intermediates for the synthesis of O-linked glycopeptides. Efficient β- and α-selective glycosylations of serine and threonine residues have been accomplished using the classical Koenigs–Knorr reaction and Lemieux's in situ anomerization methodology. Purification of the glycosyl Schiff bases is straightforward. Deprotection of these Schiff base glycosides has been accomplished using mildly acidic conditions or hydrogenolysis. Solution-phase and solid-phase peptide syntheses have been demonstrated using the glycosyl Schiff bases as starting materials. This methodology should be amenable to the synthesis of serine/threonine glycosides via block-type transfers of polysaccharide donors due to the mild conditions required for glycosylation of the Schiff bases.

Experimental Section

General Methods. Resins and Fmoc-amino acids were purchased from Bachem California (Torrance, CA). All air- and moisture-sensitive reactions were performed under an argon atmosphere in flame-dried reaction flasks. THF was dried and deoxygenated over Ph_2O=O/Na^0–K^0. 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,[31] 400–230 mesh silica gel 60 (E. Merck No. 9385) was employed. All compounds described were >95% pure by ^1H and ^{13}C NMR, and purity was confirmed by elemental analysis in many cases. The ^1H and ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer at 250 and 62.9 MHz, respectively. COSY spectra were obtained on a Bruker WM-500 spectrometer at 500 MHz. Chemical shifts are reported in δ vs Me_4Si in ^1H spectra and vs $CDCl_3$ 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). Nominal and exact mass spectra were obtained on a JEOL JMS-01SG-2 mass spectrometer.

Diphenylmethyl *N*-(Diphenylmethylene)-L-serinate (3b). The procedure used is that of O'Donnell.[25] Diphenylmethyl L-serinate salt (**1b**) (24.39 g, 55 mmol), Ph_2C=NH (9.06 g, 50 mmol), and CH_2Cl_2 (80 mL) were stirred at room temperature for 24 h with the exclusion of moisture ($CaCl_2$ tube). The reaction mixture was diluted with CH_2Cl_2 (100 mL), filtered, and washed with 1% $NaHCO_3/H_2O$ (3 × 30 mL) to remove the precipitated NH_4Cl. The organic layer was dried ($MgSO_4$) and evaporated. The resulting mass was recrystallized from Et_2O/hexane to give 16.11 g of **3b** (74%): mp 137–9 °C; $[\alpha]^{20}_D$ = −112° (*c* = 1.0, $CHCl_3$); R_f 0.46 (hexane/EtOAc, 8:2). Anal. Calcd for $C_{29}H_{25}O_3N$: C, 79.98; H, 5.79; N, 3.22. Found: C, 79.87; H, 5.84; N, 3.10.

Benzyl *N*-(Diphenylmethylene)-L-serinate (3c). Reaction was the same as in **3b**. Recrystallization (cyclohexane) provided pure **3c** in 85% yield: mp 78 °C; $[\alpha]^{20}_D$ = −120.4° (*c* = 0.9, $CHCl_3$); R_f 0.47 (hexane/EtOAc, 81:19). Anal. Calcd for $C_{23}H_{21}O_3N$: C, 76.86; H, 5.89; N, 3.90. Found: C, 76.59; H, 5.98; N, 3.73.

Methyl *N*-(Diphenylmethylene)-L-threoninate (4a). Reaction was the same as in **3b**. Recrystallization (Et_2O/hexane) provided pure **4a** in 81% yield: mp 82.5–84.5 °C; $[\alpha]^{20}_D$ = −146° (*c* = 1.1, $CHCl_3$); R_f 0.54 (hexane/EtOAc, 7:3). Anal. Calcd for $C_{18}H_{19}O_3N$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.80; H, 6.40; N, 4.59.

Diphenylmethyl *N*-(Diphenylmethylene)-L-threoninate (4b). Reaction was the same as in **3b**. Recrystallization (Et_2O/hexane) provided **4b** in 78% yield: mp 107–9 °C; $[\alpha]^{20}_D$ = −111° (*c* 1.0, $CHCl_3$); R_f 0.24 (hexane/EtOAc, 9:1). Anal. Calcd for $C_{30}H_{27}O_3N$: C, 80.15; H, 6.05; N, 3.12. Found: C, 80.31; H, 5.97; N, 2.99.

Ethyl *N*-[*N*-(Diphenylmethylene)-L-seryl]glycinate (5). Reaction was the same as in **3b**. Recrystallization (Et_2O/hexane) provided **5** in 80% yield: mp 103–5 °C; $[\alpha]^{20}_D$ = +59.5° (*c* = 0.21, $CHCl_3$); R_f 0.18 (hexane/EtOAc, 55:45). Anal. Calcd for $C_{20}H_{22}O_4N_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.54; H, 6.22; N, 7.71.

[Methyl *N*-(diphenylmethylene)-L-serinate-3-yl] 2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside (6). Methyl *N*-(diphenylmethylene)-L-serinate[27] (**3a**) (447 mg, 1.58 mmol), acetobromoxylose[48] (**17**) (535 mg, 1.2 equiv), powdered, oven-dried 4-Å molecular sieves (1.5 g), and CH_2Cl_2 (10 mL) were stirred at 0 °C under argon for 10 min. Silver triflate (492 mg, 1.2 equiv) was added in portions over 10 min, and stirring was continued for 14 h. The reaction was quenched with Et_3N (0.5 mL), diluted with CH_2Cl_2 (30 mL), and filtered through Celite, and the organic layer was washed with saturated $NaHCO_3$ (3 × 15 mL) and H_2O (3 × 15 mL) and dried ($MgSO_4$). Rotary evaporation and flash chromatography[31] on 50 g of SiO_2 with hexanes/EtOAc (6:4) (R_f 0.49) provided 804 mg of pure **6** as a syrup (94%): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −78° (*c* = 0.46, $CHCl_3$). Anal. Calcd for $C_{28}H_{31}O_{10}N$: C, 62.09; H, 5.77; N, 2.58. Found: C, 62.28; H, 5.81; N, 2.51.

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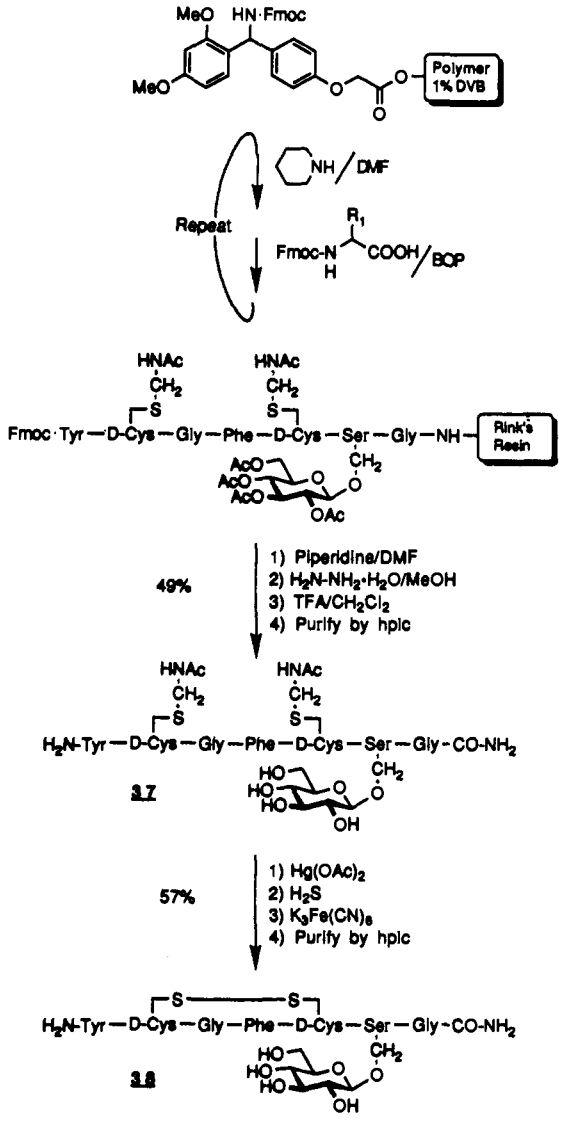
Scheme VII



[Methyl *N*-(diphenylmethylene)-L-serinate-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (7). Methyl *N*-(diphenylmethylene)-L-serinate[27] (**3a**) (1.11 g, 3.9 mmol), acetobromoglucose (**18**) (1.93 g, 1.2 equiv), powdered, oven-dried 4-Å molecular sieves (2.0 g), and CH_2Cl_2 (20 mL) were stirred at 0 °C under argon for 10 min. Silver triflate (1.2 g, 1.2 equiv) was added in portions over 20 min, and stirring was continued for 14 h. The reaction was quenched with Et_3N (0.7 mL), diluted with CH_2Cl_2 (60 mL), and filtered through Celite, and the organic layer was washed with saturated $NaHCO_3$ (3 × 15 mL) and H_2O (3 × 15 mL) and dried ($MgSO_4$). Rotary evaporation and flash chromatography[31] on 100 g of SiO_2 with hexanes/EtOAc (55:45) (R_f 0.61) provided 2.01 g of pure **7** as a syrup (83.6%): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −48.0° (*c* = 0.82, $CHCl_3$). Anal. Calcd for $C_{31}H_{35}O_{12}N$: C, 60.67; H, 5.74; N, 2.28. Found: C, 60.49; H, 5.82; N, 2.19.

[Methyl *N*-(diphenylmethylene)-L-serinate-3-yl] 2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-D-lactoside (8). Method A (Modified Koenigs–Knorr[30] Reaction). Reaction was the same as in **3a** → **7** above, using **3a** (849 mg, 3.0 mmol), and acetobromolactose[49] (**20**) (2.52 g, 1.2 equiv) to provide 2.02 g of **8** as a foam (88%) after chromatography[31] (R_f 0.8 CH_2Cl_2/acetone, 87:13): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −29.7° (*c* = 1.26, $CHCl_3$). Anal. Calcd for $C_{43}H_{51}O_{20}N$: C, 57.26; H, 5.70; N, 1.55. Found: C, 57.41; H, 5.58; N, 1.57.

Method B (Kochetkov[52] Method). Orthoester **22** (626 mg, 1.0 equiv), Schiff base **3a** (59 mg, 0.30 equiv), powdered, oven-dried 4-Å molecular sieves (1.5 g), and CH_2Cl_2 (10 mL) were stirred at room temperature under argon for 10 min. Silver triflate (178 mg, 1.0 equiv) was added into this mixture, and stirring was continued overnight. Workup as in **3a** → **7** above provided 395 mg of **8** (63%).

[Diphenylmethyl *N*-(diphenylmethylene)-L-serinate-3-yl] 3,4,6-Tri-*O*-acetyl-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-β-D-glucopyranoside (9). Reaction was the same as in **3a** → **7** above, using **3b** (449.5 mg, 1.00 mmol) and bromide **21**[50] (815.4 mg, 1.5 equiv) to provide 747 mg of **9** as a foam (81%) after chromatography[31] (R_f 0.43 hexene/EtOAc, 6:4): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −7.9° (*c* = 0.9, $CHCl_3$). Anal. Calcd for $C_{44}H_{43}O_{12}N_2Cl_3$: C, 58.84; H, 4.83; N, 3.12. Found: C, 58.49; H, 4.97; N, 3.01.

[Diphenylmethyl *N*-(diphenylmethylene)-L-serinate-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (10). Reaction was the same as in **3a** → **7** above, using **3b** to provide **10** in 77% yield as a syrup after chromatography[31] (R_f 0.5 hexanes/EtOAc, 6:4): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −24° (*c* = 0.6, $CHCl_3$). Anal. Calcd for $C_{43}H_{43}O_{12}N$: C, 67.43; H, 5.65; N, 1.82. Found: C, 67.27; H, 5.82; N, 1.69.

[Diphenylmethyl *N*-(diphenylmethylene)-L-serinate-3-yl] 2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-D-lactoside (11). Reaction was the same as in **3a** → **7** above, except 1.0 equiv of acceptor **3b**, 1.6 equiv of donor **20**, and 1.6 equiv of $AgSO_3CF_3$ were used to provide **11** as a foam in 79% yield after chromatography[31] (R_f 0.53 hexanes/EtOAc, 45:55): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −19.5° (*c* = 0.65, $CHCl_3$). Anal. Calcd for $C_{55}H_{59}O_{20}N$: C, 62.67; H, 5.64; N, 1.33. Found: C, 62.74; H, 5.58; N, 1.21.

[Methyl *N*-(diphenylmethylene)-L-threoninate-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (12) and 1,2-*O*-[[[Methyl *N*-(diphenylmethylene)-L-threoninate-3-yl]oxy]ethylidyne]-3,4,6-tri-*O*-acetyl-α-D-glucopyranose (12a). Reaction was the same as in **3a** → **7** above, using Schiff base **4a** (892 mg, 3.00 mmol) and acetobromoglucose (**18**) (1.60 g, 1.3 equiv) to provide 1.20 g of **12** as a syrup (64%) after chromatography[31] (R_f 0.34 hexanes/EtOAc, 65:35): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −72.8° (*c* = 0.9, $CHCl_3$). Anal. Calcd for $C_{32}H_{37}O_{12}N$: C, 61.23; H, 5.94; N, 2.31. Found: C, 61.40; H, 6.02; N, 2.21.

Orthoester **12a** (R_f 0.54) was also isolated (330 mg, 17%): mp 115–116 °C (recrystallized from hexanes/EtOAc); $[\alpha]^{20}_D$ = −38° (*c* = 0.34, $CHCl_3$); characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.66–7.15 (m, 10 H, aromatic H), 5.60 (d, 1 H, H-1, $J_{1,2}$ = 4.2 Hz), 3.68 (s, 3 H, OCH_3), 2.09, 2.08, 2.02 (3s, 9 H, 3O=CCH_3), 1.69 (s, 3 H, orthoester CH_3), 1.19 (d, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 121.37 (orthoester quaternary C), 96.64 (C1), 63.03 (C6), 51.85 (OCH_3), 21.62 (orthoester CH_3), 18.50 (CH_3). Anal. Calcd for $C_{32}H_{37}O_{12}N$: C, 61.23; H, 5.94; N, 2.31. Found: C, 61.22; H, 5.89; N, 2.17.

[Methyl *N*-(diphenylmethylene)-L-threoninate-3-yl] 2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-D-lactoside (13). Reaction as in **3a** → **7** above, using **4a** (892 mg, 3.00 mmol) and **20** (2.72 g, 1.3 equiv) to provide 2.23 g was the same **13** as a foam (81%) after chromatography[31] (R_f 0.40 hexanes/EtOAc, 45:55): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −37° (*c* = 0.96, $CHCl_3$). Anal. Calcd for $C_{44}H_{53}O_{20}N$: C, 57.69; H, 5.83; N, 1.52. Found: C, 57.82; H, 5.89; N, 1.38.

[Diphenylmethyl *N*-(diphenylmethylene)-L-threoninate-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (14). Reaction was the same as in **3a** → **7** above to give **14** from **4b** and **18** in 45% yield as a foam after chromatography[31] (R_f 0.52 hexanes/EtOAc, 6:4): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −49.5° (*c* = 2.3, $CHCl_3$). Anal. Calcd for $C_{44}H_{45}O_{12}N$: C, 67.77; H, 5.82; N, 1.80. Found: C, 67.91; H, 5.66; N, 1.67.

[Diphenylmethyl *N*-(diphenylmethylene)-L-threoninate-3-yl] 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranoside (15). Reaction was the same as in **3a** → **7** above to give **15** from **4b** and **19** in 63% yield as a foam after chromatography[31] (R_f 0.47 hexanes/EtOAc, 7:3): for ^1H and ^{13}C NMR data, see Tables I and II; $[\alpha]^{20}_D$ = −2.0° (*c* = 1.2, $CHCl_3$). Anal. Calcd for $C_{64}H_{53}O_{12}N$: C, 74.77; H, 5.20; N, 1.36. Found: C, 74.62; H, 5.34; N, 1.21.

***N*-(Diphenylmethylene)-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)-(1→3)-L-seryl-L-glycine Ethyl Ester (16).** Coupling as described for **7** gave **16** in 86% yield as an amorphous solid after chromatography[31] (R_f 0.6 hexanes/EtOAc, 25:75): $[\alpha]^{20}_D$ = −6.2° (*c* = 0.64, $CHCl_3$); characteristic ^1H NMR data, ^1H NMR ($CDCl_3$) δ 7.70–7.14 (m, 10 H, aromatic H), 4.46 (d, 1 H, H-1, $J_{1,2}$ = 7.6 Hz), 4.39 (d, 1 H, H-1′, $J_{1',2'}$ = 7.8 Hz), 2.14–1.94 (7s, 21 H, 7O=CCH_3), 1.28 (t, 3 H, CH_2CH_3); for ^{13}C NMR data, see Table II. Anal. Calcd for

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$C_{46}H_{56}O_{21}N_2$: C, 56.78; H, 5.80; N, 2.87. Found: C, 56.57; H, 5.68; N, 2.78.

1,2-*O*-[[[Methyl *N*-(diphenylmethylene)-L-serinate-3-yl]oxy]-ethylidyne]-3,6-di-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-α-D-glucopyranose (22). A mixture of acetobromolactose (**20**) (435 mg, 0.62 mmol), Schiff base **3a** (180 mg, 0.636 mmol), and $NaHCO_3$ (185 mg, 2.2 mmol) was stirred in THF (12 mL) at 0 °C for 10 min. Then a solution of AgOTfl (183 mg in 8 mL of THF) was added dropwise. After 10 min the reaction mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was separated, washed with cold H_2O and saturated $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was chromatographed[31] (R_f 0.25 hexanes/EtOAc, 55:45) and recrystallized (Et_2O/pentane) to give 250 mg of **22** (45%): mp 93–95 °C; $[\alpha]^{20}_D = -35°$ (c = 0.21, $CHCl_3$); characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.65–7.15 (m, 10 H, aromatic H), 5.58 (d, 1 H, H-1, $J_{1,2}$ = 4.6 Hz), 4.51 (d, 1 H, H-1′, $J_{1',2'}$ = 7.9 Hz), 3.73 (s, 3 H, OCH_3), 2.18–1.96 (6s, 18 H, 6O=CCH_3), 1.68 (s, 3 H, orthoester CH_3); ^{13}C NMR (C_6D_6) δ 121.77 (orthoester quaternary C), 103.14 (C1′), 97.22 (C1), 78.47 (C4), 64.16 (C6), 60.98 (C6′), 51.61 (OCH_3). Anal. Calcd for $C_{43}H_{51}O_{20}N$: C, 57.26; H, 5.70; N, 1.55. Found: C, 57.29; H, 5.79; N, 1.48.

1,2-*O*-[[[Methyl *N*-(diphenylmethylene)-L-threoninate-3-yl]oxy]-ethylidyne]-3,6-di-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-α-D-glucopyranose (23). Reaction was the same as in **3a** → **22** above, with Schiff base **4a** and acetobromolactose (**20**) reacting to give **23** in 55% yield after chromatography[31] (R_f 0.35 hexanes/EtOAc, 1:1) and recrystallization (hexanes/EtOAc): mp 158–160 °C; $[\alpha]^{20}_D = -29°$ (c = 0.5, $CHCl_3$); characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.66–7.15 (m, 10 H, aromatic H), 5.53 (d, 1 H, H-1, $J_{1,2}$ = 4.9 Hz), 4.58 (d, 1 H, H-1′, $J_{1',2'}$ = 8.0 Hz), 3.68 (s, 3 H, OCH_3), 2.17–1.97 (6s, 18 H, 6O=CCH_3), 1.69 (s, 3 H, orthoester CH_3), 1.20 (d, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 121.40 (orthoester quaternary C), 101.92 (C1′), 96.49 (C1), 77.37 (C4), 63.21 (C6), 60.74 (C6′), 51.72 (OCH_3), 21.30 (orthoester CH_3), 18.42 (CH_3). Anal. Calcd for $C_{44}H_{53}O_{20}N$: C, 57.69; H, 5.83; N, 1.52. Found: C, 57.77; H, 5.72; N, 1.41.

1,2-*O*-[[[Methyl *N*-(diphenylmethylene)-L-serinate-3-yl]oxy]-ethylidyne]-3,4,6-tri-*O*-acetyl-α-D-glucopyranose (24). Reaction was the same as in **3a** → **22** above, with Schiff base **3a** and acetobromoglucose (**18**) giving **24** in 52% yield after chromatography[31] (R_f 0.2 hexanes/EtOAc, 7:3) and recrystallization (Et_2O/pentane): mp 49–51 °C; $[\alpha]^{20}_D = -35°$ (c = 0.4, $CHCl_3$); characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.64–7.18 (m, 10 H, aromatic H), 5.61 (d, 1 H, H-1, $J_{1,2}$ = 5.2 Hz), 3.71 (s, 3 H, OCH_3), 2.09, 2.08, 2.04 (3s, 9 H, 3O=CCH_3), 1.67 (s, 3 H, orthoester CH_3); ^{13}C NMR ($CDCl_3$) δ 120.93 (orthoester quaternary C), 96.61 (C1), 64.29 (β-C), 62.94 (C6), 52.12 (OCH_3). Anal. Calcd for $C_{31}H_{35}O_{12}N$: C, 60.67; H, 5.74; N, 2.28. Found: C, 60.71; H, 5.89; N, 2.20.

Methyl *N*-(Benzyloxycarbonyl)-*N*-methyl-L-serinate (25). *N*-Methyl-L-serine (Schweizerhall Inc.) (800 mg) was suspended in dry MeOH (8 mL) and cooled to −10 °C. Into this solution was dropped $SOCl_2$ (0.64 mL) with vigorous stirring. After the mixture was refluxed for 4 h, the product was triturated in dry Et_2O and filtered. The resulting precipitate was suspended in dry CH_2Cl_2 (12 mL) and cooled to 0 °C. Et_3N (2.8 mL) and $PhCH_2OCOCl$ (0.9 mL) were dropped simultaneously. The stirring was continued for 14 h at room temperature, and the reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with 0.5 N HCl (3 × 10 mL) and H_2O, dried ($MgSO_4$), and filtered. Chromatography[31] (R_f 0.5 hexanes/EtOAc, 4:6) provided 1.31 g of **25** as a syrup (73.1%): characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.36–7.26 (m, 5 H, aromatic H), 5.15, 5.11 (s, 2 H, CH_2Ph), 4.61, 4.49 (t, 1 H, α-H), 4.07, 3.95 (m, 2 H, β-H), 3.75, 3.62 (s, 3 H, OCH_3), 2.98 (s, 3 H, NCH_3), 2.82, 2.49 (bt, 1 H, OH). Anal. Calcd for $C_{13}H_{17}O_5N$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.29; H, 6.60; N, 5.05.

[Methyl *N*-(benzyloxycarbonyl)-*N*-methyl-L-serinate-3-yl] 2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-D-lactoside (25a). Reaction was the same as in **3a** → **7** above, with **25** and **20** giving **25a**) as a foam (40%) after chromatography[31] (R_f 0.41 hexanes/EtOAc, 4:6): $[\alpha]^{20}_D = -18°$ (c = 0.7, $CHCl_3$); characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.37–7.26 (m, 5 H, aromatic H), 4.534 (d, 1 H, H-1, $J_{1,2}$ = 7.8 Hz), 4.472 (d, 1 H, H-1′, $J_{1',2'}$ = 7.7 Hz), 2.15–1.98 (m, 21 H, 7O=CCH_3). Anal. Calcd for $C_{39}H_{51}O_{22}N$: C, 52.87; H, 5.80; N, 1.58. Found: C, 52.94; H, 5.69; N, 1.47.

Methyl *N*-(Benzyloxycarbonyl)-L-serinate (26). Methyl L-serinate·HCl (2.33g) was suspended in dry CH_2Cl_2 (30 mL) and cooled to 0 °C. Into this mixture were dropped Et_3N (7.5 mL) and benzyloxy chloroformate (2.15 mL) carefully, at the same time. The stirring was continued for 16 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed by 0.5 N HCl solution (3 × 10 mL) and water, dried by $MgSO_4$, filtered, and separated on a column to give **26**: 2.4 g (63.6%); (R_f 0.35 hexanes/EtOAc, 1:1); ^1H NMR ($CDCl_3$) δ 7.36–7.26 (m, 5 H, aromatic H), 5.79 (bd, 1 H, NH), 5.12 (s, 2 H, CH_2), 4.44 (bm, 1 H, α-H), 3.94 (bm, 2 H, β-H), 3.77 (s, 3 H, OCH_3), 2.47 (bt, 1 H, OH). Anal. Calcd for $C_{12}H_{15}O_5N$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.72; H, 6.09; N, 5.31.

Competition Reaction between 3a and 26. Two equivalents each of **3a** and **26** were treated with 1 equiv of acetobromoglucose (**18**) and 1 equiv of $AgSO_3CF_3$ as described for **22**. Orthoester **24** was the only carbohydrate-bearing substance observed in the crude reaction mixture and was identified by the characteristic signals of its ^1H NMR spectrum: ^1H NMR ($CDCl_3$) δ 7.64–7.18 (m, 10 H, aromatic **H**), 5.61 (d, 1 H, H-1, $J_{1,2}$ = 5.2 Hz), 3.71 (s, 3 H, OCH_3), 2.09, 2.08, 2.04 (3s, 9 H, 3O=CCH_3), 1.67 (s, 3 H, orthoester CH_3).

[Methyl *N*-(benzyloxycarbonyl)-L-serinate-3-yl] 2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-D-lactoside (26a). Reaction was the same as in **3a** → **7** above, with **26** and **20** giving **26a** as a foam (18%) after chromatography[31] (R_f 0.39 hexanes/EtOAc, 4:6). Characteristic NMR data: ^1H NMR ($CDCl_3$) δ 7.38–7.26 (m, 5 H, aromatic H), 5.79 (broad d, 1 H, NH), 4.45 (d, 1 H, H-1, $J_{1,2}$ = 7.9 Hz), 4.43 (d, 1 H, H-1′, $J_{1',2'}$ = 7.9 Hz), 3.74 (s, 3 H, OCH_3), 2.15–1.96 (7s, 21 H, 7O=CCH_3). Anal. Calcd for $C_{38}H_{49}O_{22}N$: C, 52.35; H, 5.67; N, 1.61. Found: C, 52.51; H, 5.44; N, 1.47.

[Methyl *N*-(diphenylmethylene)-L-serinate-3-yl] 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranoside (28). Schiff base **3a** (283 mg, 1 mmol), $nBu_4N^+Br^-$ (644 mg, 2 equiv) powdered, oven-dried 4-Å molecular sieves (2.5 g) were stirred in 10 mL of dry CH_2Cl_2 at room temperature under argon. After 10 min of stirring, glycosyl donor **27**[36] (580 mg, 0.96 mmol) and iPr_2NEt (193 mg, 1.5 equiv) were added. After 7 days, the ratio of bromo sugar to product (~2:8) was no longer changing by TLC. The mixture was diluted with CH_2Cl_2 (50 mL) and filtered through Celite, and the organic layer was washed with H_2O (3 × 15 mL) and dried ($MgSO_4$). Rotary evaporation and flash chromatography[31] (R_f 0.49 hexanes/EtOAc/CH_2Cl_2, 7:2:1) provided 410 mg of pure **28** as a syrup (53%): $[\alpha]^{20}_D = +38°$ (c = 0.40, $CHCl_3$). Anal. Calcd for $C_{51}H_{51}O_8N$: C, 76.00; H, 6.37; N, 1.73. Found: C, 75.83; H, 6.29; N, 1.66.

[Methyl L-serinate-3-yl] α-D-Glucopyranoside Hydrochloride (29). Glycoside **28** (25 mg) was dissolved in MeOH (5 mL), and HCl (2.6 μL, 36%) and 5% palladium on activated carbon (25 mg) were added. The mixture was stirred under H_2 (balloon) for 2 h, diluted with CH_2Cl_2 (20 mL), filtered, and evaporated to give 9.5 mg of **29** as an amorphous solid (96%): $[\alpha]^{20}_D = +21°$ (c = 1.1, CH_3OH); characteristic NMR data, ^1H NMR (D_2O) δ 4.72 (d, 1 H, H-1, $J_{1,2}$ = 3.7 Hz), 3.65 (s, 3 H, OCH_3); for ^{13}C NMR data, see Table II. Anal. Calcd for $C_{10}H_{20}O_8NCl$: C, 37.80; H, 6.34; N, 4.41. Found: C, 37.51; H, 6.17; N, 4.18.

[Benzyl *N*-(diphenylmethylene)-L-serinate-3-yl] 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranoside (30). Reaction was the same as in **3a** → **28** above, with Schiff base **3c** and glycosyl donor **27** giving **30** as a foam (56%) after chromatography[31] (R_f 0.33 hexane/EtOAc, 8:2): $[\alpha]^{20}_D = -17.1°$ (c = 0.9, $CHCl_3$); for ^{13}C NMR data, see Table II. Anal. Calcd for $C_{57}H_{55}O_8N$: C, 77.62; H, 6.28; N, 1.59. Found: C, 77.91; H, 6.10; N, 1.47.

[Methyl L-serinate-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (31). Glycoside **7** (200 mg) was stirred in THF (4.5 mL), and CF_3CO-OH (0.5 mL) was dropped into it. After 5 min the reaction was complete. The mixture was evaporated, and the residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$ (3 × 10 mL) and H_2O (3 × 10 mL), and dried ($MgSO_4$). Solvent removal provided a syrup which was chromatographed on a short column[31] (R_f 0.61 CH_2Cl_2/MeOH 93:7) to provide 135 mg of **31** as a syrup (92%): $[\alpha]^{20}_D = -12°$ (c = 0.4, $CHCl_3$); for ^1H and ^{13}C NMR data, see Tables I and II. Anal. Calcd for $C_{18}H_{27}O_{12}N$: C, 48.10; H, 6.05; N, 3.11. Found: C, 48.27; H, 6.14; N, 3.02.

***N*-(*tert*-Butyloxycarbonyl)-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactosyl)-(1→3)-L-seryl-L-glycine Ethyl Ester (32).** Solid Na_2CO_3 (35 mg), t-Boc_2O (62 mg), 5% Pd–C (220 mg), and glycoside **16** (220 mg) were stirred in EtOAc (10 mL) under H_2 (balloon) at room temperature. After 4 h the starting material had been consumed, and the H_2 was removed by vacuum, the Pd–C was filtered off, and the solution was evaporated and chromatographed on a short column[31] (R_f 0.6 hexanes/EtOAc, 25:75) to give 180 mg of **32** as a syrup (86%): $[\alpha]^{20}_D = +4.3°$ (c = 1.2, $CHCl_3$); characteristic ^1H NMR data, ^1H NMR ($CDCl_3$) δ 4.596 (d, 1 H, H-1, $J_{1,2}$ = 7.8 Hz), 4.502 (d, 1 H, H-1′, $J_{1',2'}$ = 7.8 Hz), 2.15–1.97 (7s, 21 H, 7O=CCH_3), 1.44 (s, 9 H, $C(CH_3)_3$), 1.29 (t, 3 H, CH_2CH_3); for ^{13}C NMR data, see Table II. Anal. Calcd for $C_{38}H_{56}O_{23}N_2$: C, 50.21; H, 6.21; N, 3.08. Found: C, 49.98; H, 6.16; N, 2.99.

[Diphenylmethyl L-serinate-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (33). Glycoside **10** was stirred in THF and CF_3COOH as in **7** → **31** above and chromatographed on a short column[31] (R_f 0.41 CH_2Cl_2/EtOAc/MeOH, 85:10:5) to give **33** (81%): $[\alpha]^{20}_D = -11.7°$ (c = 0.75, $CHCl_3$); for ^1H NMR data, see Table I. Anal. Calcd for

$C_{30}H_{35}O_{12}N$: C, 59.89; H, 5.86; N, 2.32. Found: C, 59.71; H, 5.94; N, 2.22.

(L-Serin-3-yl) 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (34). Glycoside **10** (25 mg) and 5% Pd-C (25 mg) were stirred in MeOH (5 mL) under H_2 (balloon) at room temperature. After 1.5 h the Pd-C was filtered off, and the residue was evaporated to give 14 mg of **34** (98%): mp 154 °C (recrystallized from $Me_2C{=}O$); $[\alpha]^{20}_D = -15°$ (c = 0.28, MeOH); TLC R_f 0.48 (CH_2Cl_2/MeOH, 65:35); for ^1H and ^{13}C NMR data, see Tables I and II. Anal. Calcd for $C_{17}H_{25}O_{12}N$: C, 46.71; H, 5.78; N, 3.06. Found: C, 46.48; H, 5.70; N, 2.99.

[Diphenylmethyl *N*-[*N*-(*tert*-butyloxycarbonyl)-L-phenylalanyl]-L-serinate-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (35). Amino ester glycoside free base **33** (114 mg), HOBT (25.5 mg), and *N*-α-*t*-Boc-L-Phe (50 mg) were dissolved in dry THF (5 mL) and cooled to 0 °C, and DCCI (41 mg) was added. After the mixture was stirred overnight, the precipitate was filtered, and the solution was evaporated. The residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$ and H_2O, dried ($MgSO_4$), and chromatographed on a short column[31] (R_f 0.52 CH_2Cl_2/EtOAc, 8:2) to give 136 mg of **35** as a foam (84%): $[\alpha]^{20}_D = -3.1°$ (c = 0.38, $CHCl_3$); characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.31-7.16 (m, 15 H, aromatic H), 6.82 (s, 1 H, $CHPh_2$), 2.04-2.00 (4s, 12 H, $4O{=}CCH_3$), 1.34 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 100.71 (C1), 79.86 ($C(CH_3)_3$), 61.53 (C6), 28.06 ($C(CH_3)_3$); FAB-MS (glycerin matrix) calcd monoisotopic mass for $C_{44}H_{52}O_{15}N_2$ 848.33, m/z 849.25 [MH^+].

[*N*-(9-Fluorenylmethoxycarbonyl)-L-serin-3-yl] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (36). Amino acid glycoside **34** (331 mg) was stirred in a mixture of 10% $NaHCO_3$ in water (5 mL) and dioxane (3 mL) at 0 °C. Fmoc-Cl (197 mg) in dioxane (3 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 evaporated, dissolved in CH_2Cl_2 (60 mL), washed with H_2O (3 × 10 mL), dried, evaporated, and chromatographed[31] (R_f 0.67 CH_2Cl_2/MeOH, 8:2) to give 295 mg of **36** as a foam (92%): $[\alpha]^{20}_D = +25°$ (c = 0.4, $CHCl_3$); characteristic NMR data, ^1H NMR ($CDCl_3$) δ 7.79-7.26 (m, 8 H, aromatic H), 5.66 (bd, 1 H, NH), 4.51 (d, 1 H, H-1, $J_{1,2}$ = 7.1 Hz), 3.66 (ddd, 1 H, H-5), 2.09-2.01 (4s, 12 H, $4O{=}CCH_3$). Anal. Calcd for $C_{32}H_{35}O_{14}N$: C, 58.44; H, 5.36; N, 2.12. Found: C, 58.30; H, 5.44; N, 2.01.

H_2N-Tyr-D-Cys-Gly-Phe-D-Cys-Ser(*O*-β-D-Glc)-Gly-$CONH_2$ (38). Peptide assembly via Fmoc chemistry was performed manually (0.85-g scale, 10-mL wash volumes), starting with 4-[[(2′,4′-dimethoxyphenyl)(9-fluorenylmethoxycarbonyl)amino]methyl]phenoxy resin (Rink's[18a] resin, Bachem California) (0.46 mmol/g). Fmoc removal was accomplished with piperidine/DMF (3:7, 3 × 10 min), followed by washing with DMF (6 × 1 min). Couplings were achieved by adding the reagents sequentially to the resin in the following order: 1.5 equiv of Fmoc amino acid, 1.5 equiv of BOP reagent, 1.5 equiv of HOBT, and 2.5 equiv of iPr_2NEt. The mixture was agitated by bubbling argon through the reaction mixture (frit) for 1-2 h (negative ninhydrin test obtained). Upon completion of chain assembly, the Fmoc group was removed as above. The acetyl protecting groups for the glucoside moiety were removed with $H_2NNH_2{\cdot}H_2O$/MeOH (4:1, 2 h) while the peptide remained anchored to the resin. The excess H_2NNH_2 was washed with MeOH (4 × 1 min) and CH_2Cl_2 (4 × 1 min). The cleavage was carried out with $CF_3COOH/CH_2Cl_2/H_2O$ (8:16:1, 100 mL), which also removed the *tert*-butyl ether from the side chain of the tyrosine. The filtrate was diluted with H_2O (100 mL) and vacuum distilled at 25 °C to a volume of 100 mL. After lyophilization, the crude glycopeptide was dissolved in water (20 mL), the acidity was adjusted to pH 4 with AcOH, and $Hg(OAc)_2$ (140 mg) was added. After stirring for 75 min, the reaction was diluted with H_2O (180 mL) and treated with H_2S. The HgS precipitate was removed by filtration, and excess H_2S was purged with a stream of N_2. The peptide was oxidized with aqueous $K_3Fe(CN)_6$ (312 mg in 1000 mL of H_2O) while the acidity was kept constant (pH 8.4) with NH_4OH. After 10 h the acidity was adjusted to pH 4 with AcOH, Amberlite 68 resin (Cl^- form) was added, and the reaction was stirred until the yellow color disappeared. After filtration the reaction was lyophilized and purified by HPLC (t_R = 24.9 min, 0-50% MeCN in 0.1% aqueous TFA in 50 min, C_{18} column) to provide enkephalin analogue **38** in 28% yield based on resin: characteristic ^1H NMR data, ^1H NMR (D_2O) δ 7.33-7.23 (m, 5 H, Phe aromatic H), 7.12, 6.84 (dd, 4 H, Tyr aromatic H), 4.40 (d, 1 H, H-1, $J_{1,2}$ = 7.8 Hz); FAB-MS (glycerol matrix) calcd monoisotopic mass for $C_{37}H_{50}O_{14}N_8S_2$ 894.28, m/z 895.56 [MH^+].

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Investigation of the Modes of Solubilization and Norrish II Photoreactivity of 2- and *sym-n*-Alkanones in the Solid Phases of *n*-Heneicosane and Two Homologues[1]

Alberto Nuñez,[†] George S. Hammond, and Richard G. Weiss*

Contribution from the Department of Chemistry, Georgetown University, Washington, D.C. 20057.
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Abstract: The nature of the solubilization sites and the solubility limits of a homologous series of 2- and *sym-n*-alkanones (**2-*N*** and ***m*-*N***, respectively) have been investigated in the hexagonally and orthorhombically packed layered solid phases of heneicosane (**C21**) by differential scanning calorimetry, deuterium magnetic resonance, and optical microscopy. The photoselectivity and relative quantum efficiencies of product formation from the alkanones in the same solid phases were determined. Results from experiments employing the solid phases of eicosane (**C20**) and hexacosane (**C26**) were also obtained. The data show that the solid phases of *n*-alkanes impose severe restrictions on the motions of the alkanones and their photochemically-generated hydroxy-1,4-biradical intermediates only when the solutes fit well within a solvent layer. In those cases, extremely large photoselectivities, larger than those from analogous smectic liquid-crystalline phases, can be achieved. However, the ability of a solid *n*-alkane phase to incorporate an alkanone of a different length is much more limited than in the smectic phases. Eutectic mixtures and phase-separated alkanone crystals are obtained in many of the systems investigated.

Introduction

The mechanistic features of Norrish Type II photoreactions of *n*-alkanones (eq 1) have been studied extensively and are well understood.[2] In isotropic media, quantum yields are rather insensitive to variations in temperature and solvent viscosity; the

[†] Present address: Department of Chemistry, Temple University, Philadelphia, PA 19122.

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