Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 12, pp. 1339–1361, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00133-2

Appraisal of a Glycopeptide Cloaking Strategy for a Therapeutic Oligopeptide: Glycopeptide Analogs of the Renin Inhibitor Ditekiren

Allen W. Harrison,[a] Jed F. Fisher,[a]* David M. Guido,[b] Sally J. Couch,[c] Judy A. Lawson,[c] Dorothy M. Sutter,[c] Mark V. Williams,[c] Garry L. DeGraaf,[c] John E. Rogers,[c] Donald T. Pals,[c] and Donald W. DuCharme[c]

Departments of [a]Medicinal Chemistry, [b]Biochemistry, and [c]Cardiovascular Diseases Research, Upjohn Laboratories, 301 Henrietta Street, Kalamazoo, MI 49007–4940, U.S.A.

Abstract—Among the limitations to the practical therapeutic oligopeptide are low oral availability, indifferent aqueous solubility, and an astonishingly efficient sequestration and biliary elimination by a multi-capacity liver transporter. Given the purposed use of *N*- and *O*- linked saccharides as functional appendages of eukaryotic peptides and proteins, a strategy of glycopeptide mimicry was examined for the oligopeptide renin inhibitor, ditekiren. The anticipation was that the saccharide would impart significant aqueous solubility, and might impact beneficially on the remaining two limitations. Execution of this approach was achieved by the removal of the (dimethylethoxy)carbonyl amino terminus of ditekiren, and its substitution by Boc-L-asparagine *N*-linked mono- and disaccharides. Potent hypotensive activity, as measured by a human renin-infused rat assay, is observed for virtually all of these structures (*N*-linked β-pyranose D-*N*-acetylglucosaminyl, D-glucosaminyl, D-*N*-acetylgalactosaminyl, D-mannosyl, D-galactosyl, D-maltosyl, D-cellobiosyl, D-chitobiosyl, but not L-fucosyl). The basis for this dramatic improvement (relative to ditekiren in the same assay) is the diversion of the peptide clearance from rapid liver biliary clearance to slower urinary clearance (Fisher, J. F.; Harrison, A. W.; Wilkinson, K. F.; Rush, B. R.; Ruwart, M. J. *J. Med. Chem.* **1991**, *34*, 3140). Guided by the human renin-infused rat hypertension assay, an evaluation of the linker-saccharide pairing was made. Loss of hypotensive activity is observed upon substitution of the Boc-L-asn by Boc-D-asn, and by removal of the Boc amino terminus of the glycopeptide. Potent hypotensive activity is preserved by replacement of the Boc-L-asn linker by succinate, malate, tartrate, and adipate linkers. With the longer adipate spacer, attachment of the saccharide to the P-3 phenylalanine — with omission of the P-4 proline — retains activity. These data suggest value to the glycopeptide guise for preserving the *in vivo* activity, and for the beneficial manipulation of pharmacodynamics, of this renin inhibitory oligopeptide. This strategy may have general applicability.

Introduction

The evolution of molecular structure in the hands of the medicinal chemist is the instinctive alteration of a molecular starting point. Given the importance of the polypeptide to *in vivo* communication, the plethora of polypeptide sequences identified by gene sequencing, and the synthetic ease of polypeptide construction, the *peptide* is a frequent starting point for this process. But for these same reasons all living organisms possess endogenous defense systems to prevent exposure to information-encoding xenobiotics such as the peptide. The limitations imposed by these defenses, with respect to the therapeutic peptide, are severe.[1] The simple oligopeptide is not orally available. Only at the dipeptide level, attained by exhaustive proteolytic degradation, is the peptide made fully available as a nutrient. The small fraction of the oligopeptides which is adsorbed inadvertently via pericellular pathways, is sequestered from the plasma to the bile by an efficient multi-ligand transporter of the liver.[2] As a consequence the unadorned oligopeptide is a poor therapeutic. Two possible transformation processes may be undertaken to redress the peptide's liabilities. In the first each peptidic segment is evaluated: the superfluous is expunged, and the essential is replaced (insofar as is possible) by a surrogate. In the second strategy the peptide is *cloaked* so as to disguise its character. Numerous possible guises are suggested by knowledge of structural biochemistry. Here we report our observations on the application of one guise, that of glycosylation, to the renin inhibitor ditekiren (**1**, Chart 1).[3]

Renin, an aspartyl protease, is an essential component of blood pressure regulation. It is charged with the specific task of liberating, from the peptide angiotensinogen, the decapeptide angiotensin I. This oligopeptide is in turn cleaved by the metalloprotease, angiotensin converting enzyme, to the vasoconstrictive hormone angiotensin II. Due to the central importance of this system in the regulation of blood pressure and fluid volume homeostasis,[4] the blockade of these proteases has been a central focus to the therapeutic amelioration of essential hypertension and other cardiovascular disorders. The potential value of renin inhibition in attaining a selectivity not possible with converting enzyme inhibition, and thereby minimizing clinical side effects, has driven numerous pharmaceutical efforts over the past decade. It has been a notoriously difficult undertaking. The appeal of renin as a therapeutic target — its single catalytic action on angiotensinogen — is the same reason for this difficulty. Renin achieves its specificity by full recognition of the exact (approximately six) amino acid sequence centered

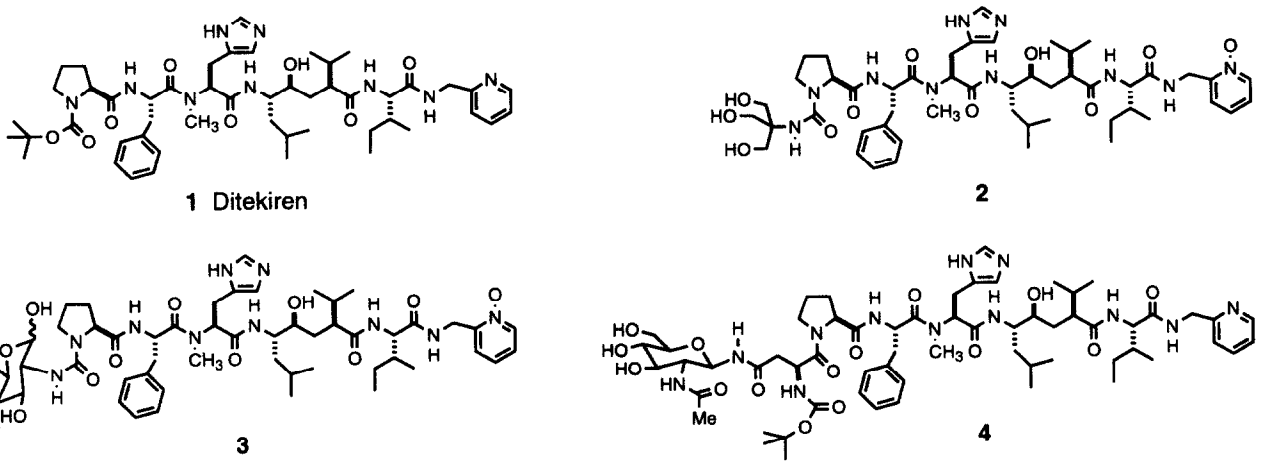


Chart 1.

about the leucine–valine scissile site. Hence the starting point for structural iteration is an oligopeptide, and the ultimate goal is an oligopeptide mimetic perceived as 'peptidic' only by renin and not at all by the xenobiotic defenses.

A benchmark entity in the Upjohn renin inhibitor effort was the ersatz heptapeptide ditekiren (**1**). Its structural relationship to the angiotensinogen cleavage site (....PFHL*VIH....) is evident. With the exceptions of the hydroxyethyl –Leuψ[CH(OH)CH_2]Val– dipeptide insert at the scissile site and the C–terminus aminomethyl pyridine mimetic of histidine, the structure is interconnected amino acid segments. The determination that ditekiren was poorly orally available (1–2% in the rat)[5a] and quickly removed from serum by the hepatic transporter,[5b,c] therefore, was of small surprise. Although the evolution of renin inhibitor structure by iterative insertion of peptide mimetic segments has proceeded remarkably — there are now several renin inhibitors for which primate and pre–clinical human data have been reported[6–13] — our approach was to identify a molecular guise for ditekiren.

The defining focus to our approach derived from a concurrent effort to improve the poor aqueous solubility of ditekiren.[13] Replacement of its N– and C–termini with water–solubilizing functional groups (respectively, the Boc– by that of a [[[2 hydroxy–1,1–bis(hydroxymethyl) ethyl]amino] carbonyl]– segment, and the pyridyl– by that of a pyridyl(*N*–oxide)– gave structure **2** (Chart 1), preserving excellent *in vitro* renin affinity and with significantly improved *in vivo* activity (evaluated in a human renin–infused rat hypertensive assay, *vide infra*).[13a,14] A companion structure in this series (**3**), having a [[(2–deoxy–D–glucos–2–yl)amino]carbonyl N–terminus, also possessed this improvement. The association of an N–terminus saccharide segment with improved *in vivo* activity, the common occurrence of N–linked (to asparagine) and O–linked (to threonine, serine) saccharides in eukaryotic proteins and peptide hormones, and the demonstrated relationship of these saccharide appendages to biological function,[15] suggested the application of this concept to ditekiren. Moreover, the elegant improvements in the synthetic methodology for the preparation and incorporation of glycosyl amino acids[16] rendered this a feasible undertaking. Hence the specific objective was the replacement of the Boc– on the proline terminus with a glycosyl amino acid mimic of the naturally occurring saccharide and amino acid pairings.

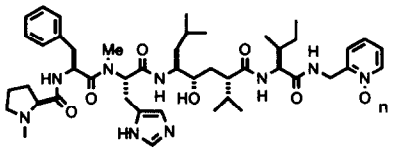
The rationale which guided this synthetic effort has been presented previously[17] and is summarized. The demands of stability and practicality were paramount. Given the probable greater intestinal instability of an O–linkage, an *N*–amide connection of the anomeric carbon of the saccharide was chosen. Although endogenous glycosyl segments can contain over a dozen saccharides in a branched array, practicality permitted only readily available mono– and disaccharides for the glycosyl segment. Use of the prolyl amine required a free carboxylate, by which to join the glycosylamide segment to the ditekiren proline. Thus the conversion of ditekiren to a glycopeptide guise required coupling of a protected glycosylamine to a monoester dicarboxylic acid, selective deprotection of the carboxylate, coupling of the glycosylamino acid to the exposed proline, and overall deprotection. The choices of saccharide and dicarboxylic acid followed from reflection upon the endogenous N–linkage. All comprise attachment of a β-Man–β–GlcNAc–β–GlcNAc– core trisaccharide to the sidechain amide of asparagine. The initial dicarboxylic acid choice was, therefore, asparagine; and the saccharide choice was that of mannose or N–acetylglucosamine if the core structure was to be emulated, or additionally saccharides such as galactose or fucose if the full gamut of antennary glycosyl segments was emulated. The initial structure prepared by this strategy (**4**), having an *N*–β–GlcNAc– (Boc)L–asn N–terminus, possessed biological activity comparable to **2** and **3**. This success prompted the assessment of the structure–activity relationship between the saccharide and dicarboxylic acid segments.

Results

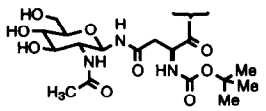
Chemistry

The ditekiren glycopeptides are divided among three

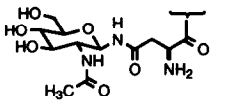
Peptidic Segment:



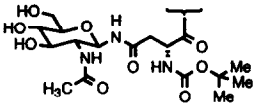
N–Terminus Saccharide Segments:



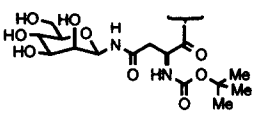
4 n = 0
9 n = 1



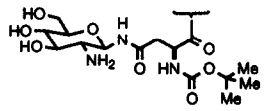
5 n = 0
6 n = 1



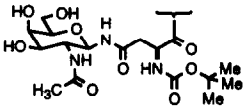
7 n = 0



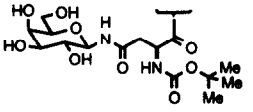
8 n = 0
10 n = 1



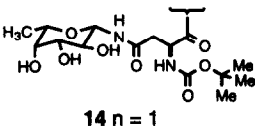
11 n = 1



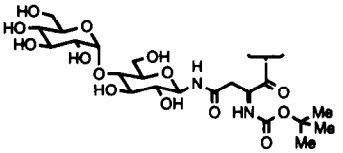
12 n = 1



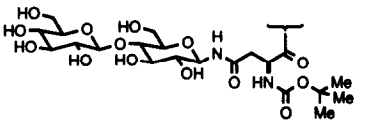
13 n = 1



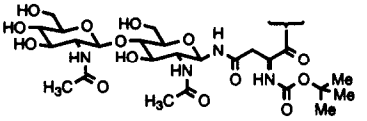
14 n = 1



15 n = 1

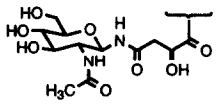


16 n = 1

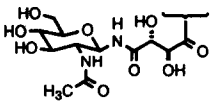


17 n = 1

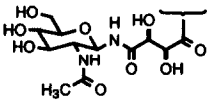
Chart 2.



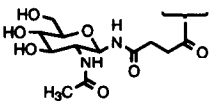
18 n = 1



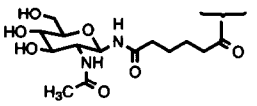
19 n = 1



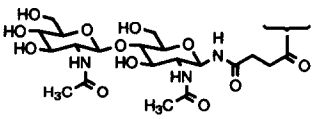
20 n = 1



21 n = 0
22 n = 1



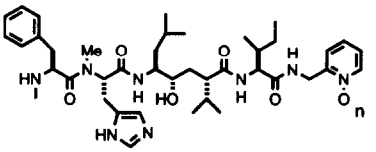
23 n = 1



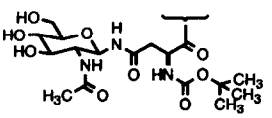
24 n = 1

Chart 3.

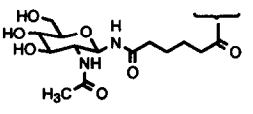
Peptidic Segment:



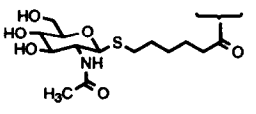
N- Terminus Saccharide Segment:



25 n = 0

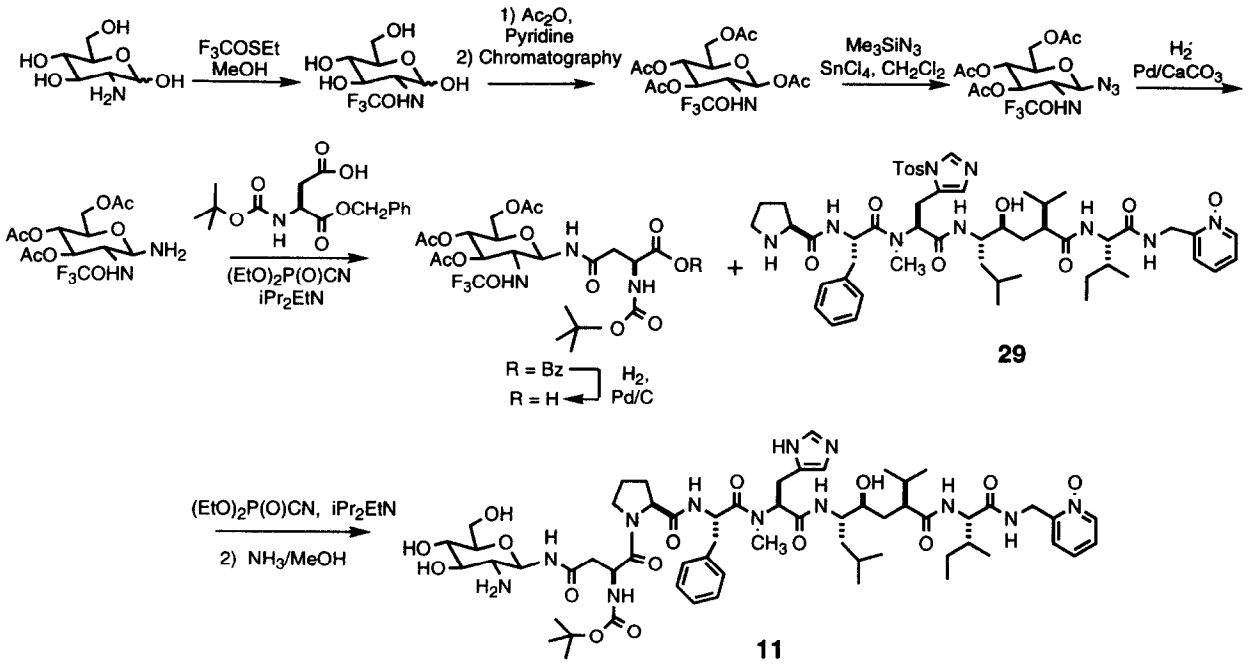


26 n = 1



27 n = 1

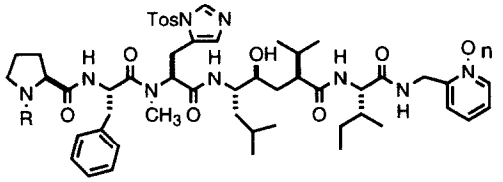
Chart 4.



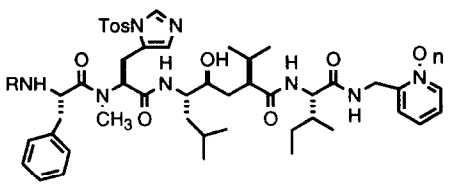
Scheme I. Synthesis of glycopeptide **11.**

categories (Charts 2–4). The first (Chart 2) contains an asparagine linker with the primary variation being saccharide structure. The second category (Chart 3) has a β-GlcNAc- (or β-GlcNAc-β-GlcNAc-) saccharide with variation in the linker structure. The third (Chart 4) comprises three structures having the saccharide–linker attached to the P-3 phenylalanine, with omission of the P-4 proline. Within these first two categories are several otherwise identical pairs of structures, differentiated at their C-terminus by the presence (or absence) of an N-oxide functionality on the pyridyl segment.

The preparation of these structures followed a straightforward sequence. Starting from the *O*-acetyl α-glycosyl halide, or *O*-acetyl β-glycosyl acetate, the β-glycosyl azide was produced by direct nucleophilic displacement by azide in hot formamide or Lewis acid–catalyzed reaction with $(CH_3)_3SiN_3$, respectively.[18] Catalytic hydrogenation (5 % $Pd/CaCO_3$) provided the β-glycosylamine, which immediately was reacted with a monobenzyl ester–protected dicarboxylic acid. Formation of the protected glycosylamide ester was accomplished by the active ester methodology now used routinely.[16,19] Standard coupling conditions, in our hands, were 1.2 equiv (relative to the glycosylamine) of the acid, 1.2 equiv iPr_2EtN or Et_3N, and 1.25 equiv of $(EtO)_2P(O)CN$. Although the yields for this coupling were often modest (40–60%), this sequence was followed due to its reliability. The preservation at the anomeric carbon of the more stable β-glycosyl configuration, established by the conditions for glycosyl azide formation, was confirmed by the ^1H NMR chemical shift and vicinal coupling constant of the anomeric carbon hydrogen (that is, in $CDCl_3$ for an *O*-acetyl *gluco* glycosyl azide, approximately δ 4.55–4.80, J = 9 Hz;[20] glycosylamine, δ 4.05–4.25, J = 9 Hz; glycosylamide, δ 5.00–5.30, J = 9 Hz).[21] Deprotection of the benzyl group (H_2, 10 % Pd/C) liberated the carboxylic acid necessary for coupling to the N-terminus of the (*N*-tosyl)imidazolyl–protected peptide (either **28, 29, 31,** or **32**). In some instances the C-terminus pyridyl *N*-oxide was installed after coupling of the glycosylamide acid to the peptide by 3-$ClPhCO_3H$ oxidation. Simultaneous removal of the two protecting groups (the *O*-acetyl on the saccharide, and *N*-tosyl on the histidinyl) was the final step in the synthesis. This was accomplished at first by saponification, but subsequently saturated methanolic NH_3 proved more convenient and reliable.[21c,22]



28 R = H; n = 0
29 R = H; n = 1



30 R = Boc; n = 0
31 R = H; n = 0
32 R = H; n = 1

Structures 28–32.

In this fashion the glycosyl Boc-L-asn linked glycopeptides **4, 9** (β-D-GlcNAc), **8, 10** (β-D-Man), **12** (β-D-GalNAc), **13** (β-D-Gal), **14** (β-L-Fuc), **15** (4-(α-D-Glc)-β-D-Glc), **16** (4-(β-D-Glc)-β-D-Glc), **17** (4-(β-D-GlcNAc)-β-D-GlcNAc) of Chart 2 were made from the initial coupling of the β-glycosylamine to the *N*-[(1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester of L-aspartic acid. The four remaining members of this set employed small variations on the standard sequence. Glycopeptides **5** and **6**, a pyridyl and pyridyl (*N*-oxide) pair, were made by CF_3CO_2H removal of the N-terminus Boc from the glycosylasparagine; the glycosyl N-linkage is stable to this treatment.[21b,23] Glycopeptide **7** was obtained from the β-GlcNAc glycosylamine and the protected D-aspartic acid. Last, the β-D-glucosaminyl glycopeptide **11** was prepared in the identical fashion as for **8**, but carrying forward *N*-trifluoroacetyl protection of the glycosyl 2-amine. This group also was removed by the final, standard saturated NH_3/MeOH deprotection, although a longer reaction time was necessary.

The remaining nine structures (six in Chart 3, and three in Chart 4) addressed practicality. As a potential therapeutic, ditekiren (**1**) was massive. The addition of a saccharide–amino acid pair exacerbated an already intolerable situation. Accordingly, the objective became simplification of structure, by removal of superfluous functionality. The obvious point of exploration was the (Boc)amino group of the asparagine; it alone adds a mass of 115 Daltons to the glycopeptide. A single saccharide, GlcNAc, was used for this series in this and the following set, as the mono- and β-disaccharide (chitobiosyl). Thus entries **21, 22** correspond to the GlcNAc-succinyl C-terminus pyridyl- and pyridyl(*N*-oxide) pair whilst **24** is the chitobiosylsuccinyl *N*-oxide. As the overall sense of the glycosylated amino terminus was that of oligohydroxyl functionalization, which as noted was of general benefit in the ditekiren series,[13a] the presence of additional hydroxyls was evaluated. These were incorporated into the spacer, first as the GlcNAc-*S*-malate (**18**), and as the GlcNAc-tartrates (**19** (*meso*-) and **20** (D-). The series of Chart 3 was completed with the GlcNAc-adipate (**23**), to evaluate tether length.

The second point of focus for mass removal was the P-4 prolyl of the peptide. A small, but perceptible, increase in inhibitor affinity is imparted by the presence of this amino acid in the ditekiren series. Renin inhibitor optimization in competitive efforts has demonstrated, however, that compensatory stabilization in much smaller peptidomimetics is possible.[6-12] An evaluation of the prolyl residue importance was made by attachment of the identical glycosylasparagine fragment (GlcNAc-Boc-L-asn) used in the synthesis of **4** and **9**, to the phenylalaninyl residue of peptide **31**. Likewise the identical fragment used for **23**, when reacted with peptide **32**, provided glycopeptide **26**. The final structure in this study (**27**), uses an alternative method for glycosyl attachment. Halide displacement from 6-bromoadipate, by the *O*-acetyl-protected GlcNAc 1-thiolate, provided the (*S*-β-D-GlcNAc)adipyl segment for reaction with **32**.

An incidental but welcome property of these glycopeptides is improved aqueous solubility. The combination in **3** of an oligoglycol N-terminus and *N*-oxide C-terminus resulted in water solubility of 100 mg mL^{-1}.[13a] A full determination of the water solubility of this glycopeptide series was not made. The solubilities (in phosphate-buffered saline) of peptides **9** (50 mg mL^{-1}), **15** (25 mg mL^{-1}), **16** (50 mg mL^{-1}), and **20** (25 mg mL^{-1}) are representative.

Biology

Ditekiren binding to human renin is kinetically complex.[3a] The K_d of the more stable renin–**1** complex was determined by Epps *et al.* to be 40 pM; the K_d for **2** was nearly identical, whereas that for **3** was an order of magnitude poorer.[3e] Determination of IC_{50} values in an *in vitro* plasma renin assay[13] gave respective values of 0.26 nM, 0.58 nM, and 1.1 nM (Table 1). In this same assay glycopeptides **4–27** — with a single outstanding exception — yielded IC_{50} values of 0.6–2.5 nM (Table 1). These values are identical within experimental error, apart from the exception (**25**, IC_{50} = 36 nM). Clearly the presence of the glycosyl asparagine segment in the prolyl binding pocket is unfavorable. A lengthened tether to the glycosyl segment (as given to **26** and **27**) provided IC_{50} values indistinguishable from those for **4–24**. Hence the evaluation of virtually this entire series may be done from the presumption of approximately equivalent renin affinity.

The primary assay used to evaluate *in vivo* performance was that of the human renin-infused, anesthetized, nephrectomized, ganglion-blocked rat devised by Pals *et al.*[14] The level of renin infusion produces a renin-dependent blood pressure component of approximately 60 mmHg that is stable during a 2 h observation period. Administration of a renin inhibitor (either iv or po) ameliorates this renin-dependent hypertension (measured as mean arterial blood pressure) in a dose-dependent fashion.[13,14] In this assay renin inhibitor **1** given in an iv dose of 0.054 μmol kg^{-1} (0.05 mg kg^{-1}), results in a maximum decrease in blood pressure of 27 mmHg. This maximal decrease is not maintained but progressively returns to the starting blood pressure. Thus, the relative effect of an inhibitor in this assay is measured firstly as the maximal blood pressure decrease, and secondly as the $t_{1/2}$ for relaxation to the starting blood pressure. The maximal renin inhibitor effect in this assay is full abolition of the renin-dependent component (–60 mmHg) sustained for the entire observation period ($t_{1/2}$ > 120 min). Relative to the 0.054 μmol kg^{-1} iv ditekiren dose, an oral dose 100-fold larger was required for comparable effect (5.4 μmol kg^{-1}, maximal decrease –24 mm Hg and $t_{1/2}$ 60 min). The dose difference is proportional to the (relatively small) portion of the oral dose which is available. Inhibitor **2** was observed to be more active than **1** at these doses, both in magnitude and duration (Table 1). Inhibitor **3**, again at these same doses, abolished a significant portion of the hypertensive component (greater than a 35 mmHg decrease) but as importantly maintained significant diminution for the entire 2 h period.

Table 1. Hypotensive activity of the glycosylated ditekiren analogs

Entry	IC_{50} *in vitro* Plasma Renin (nM)	Hypotensive Activity in Human Renin–Infused Rats[a] *i.v.* Dose (μmol kg^{-1})	Vehicle[b]	Maximal Decrease (mm Hg)	Recovery $t_{1/2}$ (min)	*p.o.* Dose (μmol kg^{-1})	Vehicle[b]	Maximal Decrease (mm Hg)	Recovery $t_{1/2}$ (min)
1[c]	0.26	0.054	citric	−25	27	5.4	citric	−24	60
2[c]	0.58	0.054	citric	−43	60	5.4	citric	−48	>120
3[c]	1.1	0.054	citric	−35	>120	5.4	citric	−39	>120
4	0.4	0.054	citric	−44	>120	5.4 5.4	citric v122	−49 −30	>120 >120
5	1.0	0.054	citric	−38	38	5.4 5.4	citric v122	−23 −22	>120 58
6	1.0	0.054	citric	−46	50	5.4	citric v122	−58 −6	71 19
7	0.6		ND[d]			5.4	v122	−18	56
8	0.6	0.054	citric	−34	>120	5.4 5.4	citric v122	−29 −28	>120 85
9	0.5	0.054	citric	−34	>120	5.4 5.4	citric v122	−42 −40	>120 >120
10	0.8		ND			5.4	v122	−54	>120
11	<2		ND			5.4	v122	−43	>120
12	<2	0.025	citric	−60	75	2.5	v122/citric	−77	>120
13	<2		ND			5.4	v122	−39	>120
14	1.9		ND			5.4	v122	−26	24
15	1.4	0.054	citric	−48	>120	5.4	v122	−35	>120
16	1.3	0.054	citric	−40	>120	5.4	v122	−38	>120
17	2.5		ND			5.4	v122	−50	>120
18[e]	<2	0.025	citric	−31	>120	2.5	v122	−38	>120
19[e]	1.3	0.011	citric	−30	60	0.11	v122	−55	>120
20[e]	1.0	0.009	citric	−40	>120	0.09	v122/citric	−51	>120
21[e]	0.6		ND			5.4	v122	−59	>120
22[e]	0.6	0.026	citric	−39	>120	0.26	v122	−37	>120
23[e]	1.2		ND			5.4	v122	−52	>120
24[e]	1.4	0.039	citric	−63	>120	0.39	v122	−75	>120
25	36	0.054	citric	−8	10	5.4	citric	−23	69
26[e]	2.2	0.006	citric	−22	>120	0.06	v122	−38	>120
27[e]	1.2	0.032	citric	−50	30	3.2	v122/citric	−46	>120

[a]Average of three animals. [b]Citric acid (0.1 M), v122 (1 % tween 80), and v122/citrate (1 % tween 80 in 0.1 M citric acid). [c]Data from Bundy *et al.*[13a] [d]ND, Not determined. [e]The doses for entries **18–24** (inclusive) and **26**, **27** are neither as large, nor sequentially consistent, as the earlier doses. The basis for the decreased dosing (and change in po/iv ratio from 100:1 to 10:1, as noted) reflect a desire for this assay to detect analogs with appreciable potency *and* oral activity. No rational explanation is possible for the (apparently) arbitrary choice of actual doses.

The objective, therefore, was an inhibitor which matched the performance of **3** but at significantly lower oral doses.

The entire glycopeptide series was evaluated in this assay (Table 1). Although a systematic comparison among these glycopeptide structures is not possible — there exist significant variations in dose and vehicle across the series, particularly for the glycopeptides of Charts 3 and 4, the overall performance of the entire series is remarkable. Of the 24 glycopeptides prepared only five were found less efficacious than **3**. The most direct presentation of the biological data is, therefore, initial comment on these five, followed by acknowl-

edgement of the more robust glycopeptides of the remaining nineteen. Glycopeptides **4** and **9** provide appropriate reference. Following iv and po dosing at the standard dose in the standard citric acid vehicle, both achieved a more than 34 mmHg decrease for the entire 120 min assay period (equal to **3**). Upon po dosing in a 1% tween 80 vehicle (v122), a decrease in both the maximal blood pressure decrease and duration was noted. The citric acid in the former vehicle is an adjuvant, increasing the portion of oligopeptide absorbed.[24] The diminished performance seen with the v122 vehicle reflects loss of this adjuvant effect, with consequent lower availability of the glycopeptide. A significant decrease in activity was seen for the pair of structures (**5** and **6**) obtained by removal of the Boc functionality from the asparagine amino terminus. A reasonable explanation is poorer metabolic stability. A curious third example is **7**, which is identical to **4** save for the D–asn configuration of the linker. It proved to be no better than **1**, and thus noticeably poorer than **4**. The basis for this difference is not obvious. A tempting speculation is access to a clearance or metabolic pathway triggered by the unnatural configuration. A similar explanation may hold as well for the fourth example, glycopeptide **14**. This poorly active member of Set 1 possesses an *N*–(L–fucosyl)(Boc)L–asn– structure. Although L–fucose is a component of the endogenous oligosaccharide structure, it is branched always from the first GlcNAc of the core trisaccharide. Its unusual placement here, and perhaps its L–configuration, may mark it for elimination or destruction (as was speculated for the D–GlcNAc–(Boc)D–asn– **7**). The fifth and final example was glycopeptide **25**, having the D–GlcNAc–(Boc)L–asn– attached to the P–3 phe of ditekiren. As discussed previously it possessed poor binding affinity for renin, and its lack of hypotensive activity was not a surprise.

The remaining 19 exhibited activity equal to or better than **2**. Particular attention is directed to five. Glycopeptide **12** ((GalNAc)–(Boc)L–asn–) at a po dose one–half that usually administered, in a v122/citric vehicle, sustained a maximal –77 mmHg blood pressure decrease. This was nearly matched (–75 mmHg) by **24** ((4–(β–D–GlcNAc)–β–D–GlcNAc)–(Boc)–L–asn–) following po dosing in v122 at a dose less than one–tenth (0.39 μmol kg^{-1}) that of the standard dose used for **3**. The magnitude of the maximal blood pressure decrease seen for these two (–75 mmHg) exceeded apparently the hypertensive component induced by the human renin–infusion (–60 mmHg). The GlcNAc–tartrates **19** and **20** achieved a greater than 50 mmHg decrease at oral doses *one–fiftieth* of the standard dose of 5.4 μmol kg^{-1}. Last, glycopeptide **26** — the least massive (M_r 1079) of the entire series — decreased the blood pressure maximally by –38 mmHg at one–ninetieth of the standard oral dose. The recovery period for each of these five was in excess of 120 min.

One of the glycopeptides (**9**) was evaluated in the conscious sodium–depleted cynomolgus monkey. The mean arterial blood pressure response following po (Table 2) and iv (Table 3) administration are compared to identical doses of peptide **2**. Although the po blood pressure decrease and duration are the same for the two within experimental error, relative to **2** the response of **9** was more variable. By comparison, for an equivalent po response a 30–fold larger dose of **1** is

Table 2. Hypotensive activity of **2** and **9** in the conscious sodium-depleted cynomolgus monkey[a] following po administration

		Mean Arterial Blood Pressure (mm Hg)					
	Dose[b]	time (min)					
Entry	(μmol kg^{-1})	0	30	60	120	180	240
Vehicle[c]	—	91 ± 4	95 ± 4	93 ± 5	91 ± 6	91 ± 6	93 ± 6
2[d]	1.5[d]	96 ± 3	93 ± 2	87 ± 3	77 ± 3	74 ± 3	75 ± 4
9	1.5[e]	104 ± 4	101 ± 4	96 ± 4	88 ± 5	86 ± 5	89 ± 5

[a]Male (4.1–7.7 kg). [b]Nasogastric tube delivery. [c]Sterile water, $n = 5$. [d]$n = 6$. [e]$n = 5$.

Table 3. Hypotensive activity of **2** and **9** in the conscious sodium-depleted cynomolgus monkey[a] following iv administration

		Mean Arterial Blood Pressure (mm Hg)					
	Dose[a]	time (min)					
Entry	(nmol kg^{-1})	0	15	30	60	120	180
Vehicle[b]	—	94 ± 3	95 ± 4	96 ± 3	94 ± 3	94 ± 3	95 ± 4
2[c]	50[c]	94 ± 2	64 ± 7	69 ± 6	75 ± 4	80 ± 4	81 ± 3
9	16[d]	88 ± 4	73 ± 7	72 ± 4	80 ± 3	87 ± 1	—
	48[e]	87 ± 5	68 ± 10	71 ± 2	72 ± 3	81 ± 2	90 ± 3

[a]Infusion over 5 min. [b]Sterile 2.5 % dextrose, $n = 5$. [c]$n = 5$. [d]$n = 4$. [e]$n = 3$.

needed. Following the 4 h observation, administration of saralasin (1 mg kg^{-1}) to the four least responders (of the set of five) with **9** resulted in a further decrease in blood pressure in three of the four animals. This indicated partial inhibition of the renin–angiotensin component. A clearer difference was seen in the iv study. At the 48 nmol kg^{-1} dose the response to **9** was both less potent and of shorter duration. The 16 nmol kg^{-1} dose of **9** gave the same initial hypotensive response as the 48 nmol kg^{-1} dose (suggesting equally full inhibition of the same renin compartment) but a response of shorter duration. There was no alteration of heart rate.

Discussion

The objective in all of the renin inhibitor efforts is the creation of an entity that matches the capability (in terms of cost of goods, potency, safety, and oral availability) of the converting enzyme inhibitors, but with amelioration of their adverse indications. Although significant progress has been made [6–12] this objective has yet to be reached. A portion of this difficulty derives from the realization that the plasma renin is a minor — and possibly even insignificant — component of blood pressure regulation.[4] It is necessary, therefore, for a renin inhibitor to access the critical renin reservoirs (possibly within the vasculature, the heart, and elsewhere) to exert its full benefit. This presents difficulty for a peptide-like structure (such as **1**) as these are cleared so quickly that penetration of the renin compartments, other than that of the plasma, does not occur in adequate concentration. It is within this understanding that the glycopeptide derivatives of **1** are assessed.

We reported previously that the increased efficacy of three of these glycopeptides (**4**, **8** and **9**) in the human renin–infused rat assay (Table 1) is a consequence of a significant elevation in serum concentration (relative to **2**).[17] This occurs as a consequence of less efficient biliary clearance by the hepatic transporter of the glycosylated structures. It may be argued (but cannot be proven) that this is a consequence of a successful 'cloaking' of the peptide: that is, mimicry of an important post-translational modification (glycosylation) disguises the actuality that these glycopeptides are xenobiotics. Since the renin component in this assay is entirely within the plasma, any structural segment which enables evasion of the biliary clearance will result in improved activity relative to **1** and **2**. The data of Table 1 establish the broad generality with regard to saccharide and spacer structure for successful evasion. *All* saccharides — with the notable exception of L-fucose — 'deceived'. Nonetheless, there appears a structure–activity relationship is present, which unfortunately is not well developed by the biological data. It must suffice to re-emphasize that the significant hypotensive activity of **19**, **20** and **26** at very low oral doses reflects either substantially greater affinity for renin than **1** or **2** (not supported by the data of Table 1) or greater resistance to biliary clearance *and* relatively greater oral availability. While the resistance to biliary clearance is easy to rationalize (although impossible to wholly attribute to glycosylation cloaking: the beneficial consequence of increasing terminus polarity, in general structural terms, is proven for **1**[13] and other renin inhibitors[7,25]) the possible increase in oral activity is not. The common sense perception of saccharides as membrane impermeable is indeed experimentally proven.[26] Nevertheless, the experience with glycosylated pro-drugs indicates that oral activity is possible.[27] A most interesting example with regard to this issue is the unusually high oral availability of a maltose-functionalized (but not with other saccharides) somatostatin.[28,29]

The similarity between **2** and **9** in the primate assay evokes but small comment. In this assay these inhibitors certainly must reach renin loci other than that of the plasma; the approximate po equivalence of the two suggest comparable ability in this respect. The several-fold poorer iv potency of **9** may be attributed to differential distribution, metabolism, to a smaller relative benefit with regard to biliary clearance, or to any number of other speculations. Indeed (and with grateful acknowledgement to a referee for the suggestions) the human renin–infused rat assay has two characteristics which may exaggerate the benefit of the glycosylation, that are not found in the primate assay. The decreased gastrointestinal motility of the anesthetized rat may prolong an oral response. Additionally the rat possesses very efficient hepatic extraction. The response in an assay having a serum target *and* sensitivity to delayed hepatic clearance (as is most certainly true for the human renin–infused rat) will be enhanced. It is regrettable that primate data are unavailable for those glycopeptides which appear distinctly superior to **9** in the rat assay.

The complex relationship between saccharide and peptide in terms of structure and biological function is a cornerstone of the emerging field of 'glycobiology'.[30,31] Although the outcome of this study is indecisive as to the full benefit of therapeutic peptide glycosylation, it reinforces emphatically that this relationship exists, and points to the possibility that glycosylation represents a strategy of generality and promise to the amelioration of the liabilities of the therapeutic oligopeptide.

Experimental

Synthesis of the glycopeptides

The syntheses of glycopeptides **1** [103336-05-6], **2** [125906-71-0], **3** [125906-77-6], **4** [137232-33-8], **8** [125907-06-4] and **9** [137232-34-9]) were described.[13a,17] Peptides **28** [125907-63-3], **30** [103372-36-7] and **31** [103818-16-2] were intermediates in the Upjohn renin effort.[13] Glycopeptides were made by the conversion of the β-D-pyranoside pentaacetate, either directly or via the pyranosylhalide, to the oligoacetyl β-D-pyranosyl azide; reduction of the azide to the amine; coupling of the amine to the protected dicarboxylic acid monoester

linker; carboxylate deprotection; coupling to the protected peptide (either **28**, **29**, **31** or **32**); and global glycopeptide deprotection. The specific intermediates are listed in order.

Preparation of glycopeptide **5**

L-Histidinamide, N-[3,4,6-tri-O-acetyl-N-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^{\alpha}$-methyl-1-[(4-methylphenyl)sulfonyl]-[1S[1R,2R*,4R*(1R*,2R*)]]* $C_{70}H_{98}N_{12}O_{18}S$. The protected, glycosylated Boc-asn glycopeptide precursor to **4** (0.40 g, 0.26 mmol) was dissolved in 2:1 CH_2Cl_2/CF_3CO_2H (6 mL) for 75 min at ambient temperature under Ar. The reaction was poured into cold satd aq. $NaHCO_3$, and extracted with three portions of CH_2Cl_2. The combined organics were concentrated, and the residue was purified by silica flash chromatography to give 0.283 g (0.20 mmol, 76 %) of the protected glycopeptide: TLC R_f 0.24 (95:5 CH_2Cl_2/5 M NH_3 in MeOH); MS (FAB) m/z 1427.7 ($[M + H]^+$, calcd 1427.7), 1274, 307, 278, 195, 154, 124.

L-Histidinamide, N-[N-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^{\alpha}$-methyl [1S[1R,2R*,4R*(1R*,2R*)]] $C_{57}H_{86}N_{12}O_{13}$.* The protected glycopeptide (0.339 g, 0.24 mmol) in MeOH (6 mL) was treated with 5 M NH_3 (2.4 mL) in MeOH. After 16 h the reaction was concentrated, and the residue was purified by silica flash chromatography to give 0.232 g (0.20 mmol, 84%) of the glycopeptide: TLC R_f 0.14 (7:3 CH_2Cl_2/5 M NH_3 in MeOH); MS (FAB) m/z 1147.649 ($[M + H]^+$, calcd 1147.652), 1129, 586, 365, 222, 204, 124, 109, 86, 70.

Preparation of glycopeptide **6**

L-Histidinamide, N-[3,4,6-tri-O-acetyl-N-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^{\alpha}$-methyl-1-[(4-methylphenyl)sulfonyl]-N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] $C_{75}H_{106}N_{12}O_{21}S$.* A solution of the protected glycopeptide (0.224 g, 0.15 mmol; penultimate structure to **5**) and *m*-$ClPhCO_3H$ (0.10 g) in $CHCl_3$ (6 mL) was stirred under Ar for 75 min. The reaction mixture was poured into 10% aq Na_2SO_3 (100 mL), and extracted with $CHCl_3$ (4 × 100 mL). The combined organics were dried, filtered, and concentrated. The residue was purified by silica flash chromatography (95:5 CH_2Cl_2:5 M NH_3 in MeOH) to yield 0.17 g (0.11 mmol, 73 %) of the protected glycoprotein *N*-oxide: TLC R_f 0.68 (9:1 CH_2Cl_2:5 M NH_3 in MeOH); MS (FAB) m/z 1543.7 ($[M + H]^+$, calcd 1543.7).

L-Histidinamide, N-[3,4,6-tri-O-acetyl-N-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^{\alpha}$-methyl-1-[(4-methylphenyl)sulfonyl]-N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] $C_{70}H_{98}N_{12}O_{19}S$.* A solution of the above protected glycopeptide *N*-oxide (0.301 g, 0.195 mmol) in 2:1 CH_2Cl_2:CF_3CO_2H (6 mL) was stirred under Ar for 1 h. The reaction mixture was poured into cold satd aq $NaHCO_3$ (100 mL), and then extracted with CH_2Cl_2 (4–125 mL). The combined organics were dried, filtered, and concentrated. The residue was purified by silica flash chromatography (95:5 CH_2Cl_2:5 M NH_3 in MeOH) to provide 0.36 g (0.25 mmol, 82%) of the product: TLC R_f 0.38 (9:1 CH_2Cl_2:5 M NH_3 in MeOH); MS (FAB) m/z 1443.7 ($[M + H]^+$, calcd 1443.7), 342, 278, 195, 150, 125.

L-Histidinamide, N-[N-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^{\alpha}$-methyl [1S[1R,2R*,4R*(1R*,2R*)]]-N-oxide $C_{57}H_{86}N_{12}O_{14}$.* A solution of the protected glycopeptide (0.35 g, 0.24 mmol) in MeOH (7 mL) was treated with 5 M NH_3 in MeOH (3 mL) for 15 h. The reaction mixture was concentrated, and the residue was purified by flash silica chromatography (70:27:3 CH_2Cl_2:MeOH:satd aq. NH_3) to yield 0.213 g (0.18 mmol, 76%) of glycopeptide **6**: TLC R_f 0.13 (70:27:3 CH_2Cl_2/MeOH/satd aq. NH_3); MS (FAB) m/z 1163.646 (calcd 1163.646), 1147, 365, 204, 124, 109, 86, 70.

Preparation of glycopeptide **7**

D-Asparagine, N^2-[(1,1-dimethylethoxy)carbonyl]-N-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-1-(phenylmethyl) ester $C_{30}H_{41}N_3O_{13}$. This material was prepared by coupling of the pyranosylamine under standard $(EtO)_2P(O)CN$ conditions. A suspension of D-aspartic acid, *N*-[1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester (0.302 g, 0.93 mmol) and 3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosylamine [4515-24-6] (0.259 g, 0.75 mmol) in CH_2Cl_2 (19 mL) were treated at ambient temperature under Ar with Et_3N (0.131 mL, 0.93 mmol) and $(EtO)_2P(O)CN$ (0.159 mL, 1.05 mmol) for 3 h. The reaction was poured into cold satd aq. $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organics were dried, filtered, and concentrated. The product was purified by flash silica chromatography (4:1 CH_2Cl_2:acetone) to provide 0.324 g (0.50 mmol, 67 %) of the protected glycoasparagine ester: TLC R_f 0.20 (4:1 CH_2Cl_2:acetone); MS (FAB) m/z 652.2738 ($[M + H]^+$, calcd 652.2717), 596, 552, 458, 330, 223, 210, 168, 150, 126, 108, 91, 57, 43.

D-Asparagine, N^2-[(1,1-dimethylethoxy)carbonyl]-N-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl] $C_{23}H_{35}N_3O_{13}$. A suspension of the above ester (0.32 g, 0.50 mmol) and 10% Pd/C (0.090 g) in

MeOH was hydrogenated at 30 psi for 3 h, and then filtered and concentrated to provide 0.29 g (0.50 mmol, 100%) of the glycosylasparagine acid: MS (FAB) m/z 562.2243 ([M + H]$^+$, calcd 562.2248), 506, 462, 368, 330, 210, 177, 168, 150, 126, 108, 57, 43.

L-Histidinamide, N-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-D-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^\alpha$-methyl-1-[(4-methylphenyl)sulfonyl]-[1S[1R,2R*,4R*(1R*,2R*)]] $C_{75}H_{106}N_{12}O_{20}S$.* The above glycoasparagine (0.277 g, 0.50 mmol) and the protected oligopeptide **28** (0.422 g, 0.51 mmol) were coupled under standard conditions, and the residue was purified by silica flash chromatography to give 0.534 g (0.39 mmol, 76 %) of the protected glycopeptide: TLC R_f 0.39 (9:1 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1527.7 [M + H]$^+$, calcd 1527.7.

L-Histidinamide, N-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-D-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^\alpha$-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] (7) $C_{62}H_{94}N_{12}O_{15}$ [125992-33-8].* The above protected glycopeptide (0.534 g, 0.39 mmol) was dissolved under Ar at ambient temperature in 4 M NH_3 in MeOH (4 mL). The solution was stirred for 18 h and then concentrated to a residue, which was purified by silica flash chromatography in 4:1 CH_2Cl_2:4 M NH_3 in MeOH to give 0.399 g (0.32 mmol, 82 %) of the glycopeptide: MS (FAB) m/z 1247.706 ([M + H]$^+$, calcd 1247.704), 586, 510, 365, 239, 222, 204, 138, 124, 109, 86, 70, 57.

*Preparation of glycopeptide **10***

2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl azide [65864-60-0] $C_{14}H_{19}N_3O_9$. A suspension of tetra-acetylmannosyl chloride (1.0 g) and NaN_3 (0.52 g) in formamide (10 mL) was stirred for 3.5 h under Ar at 80 °C, and cooled and diluted with ice water (300 mL). The suspension was extracted with $CHCl_3$, dried, and concentrated to a dark foam. The material was purified by silica flash chromatography to provide 0.55 g (1.47 mmol, 54 %) of the azide: TLC R_f 0.55 (1:1 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) : δ 5.40 (d, 1H, J = 1.8), 5.32–5.22 (m, 2H), 5.16 (dd, 1H, ω = 7 Hz), 4.31 (dd, 1H, J = 12.7, 5.8 Hz), 4.20–4.10 (m, 2H), 2.18 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 1.62 (s, 3H); MS (CI) m/z 391 ([M + NH_4]$^+$, calcd 391), 363, 336, 303, 276, 245, 168.

2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosylamine [41355-50-4] $C_{14}H_{21}NO_9$. A solution of the above azide (0.55 g, 1.47 mmol) and 5 % Pd/$CaCO_3$ (0.22 g) in abs. EtOH (17 mL) was hydrogenated at atmospheric pressure for 2.5 h. The reaction was filtered, and the filtrate evaporated to provide 0.427 g (1.23 mmol, 84 %) of the crude amine: TLC R_f 0.22 (95:5 CH_2Cl_2: MeOH); MS (FAB) m/z 348.1286 ([M + H]$^+$, calcd 348.1294), 331, 288, 271, 228, 169, 149, 127, 109.

L-Asparagine, N^2-[(1,1-dimethylethoxy)carbonyl]-N-[2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl]-1-(phenylmethyl) ester [125907-64-4] $C_{30}H_{40}N_2O_{14}$. A solution of the above amine (0.42 g, 1.21 mmol) and L-aspartic acid, *N*-[1,1-dimethylethoxy)carbonyl]-1- (phenymethyl) ester (0.468 g, 1.43 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide, after silica flash chromatography, 0.29 g (0.45 mmol, 37 %) of the glycosylasparagine ester: TLC R_f 0.30 (9:1 CH_2Cl_2:acetone); MS (FAB) m/z 653.2556 ([M + H]$^+$, calcd 653.2558), 597, 553, 417, 391, 331, 223, 169, 127, 109, 91.

L-Asparagine, N^2-[(1,1-dimethylethoxy)carbonyl]-N-[2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl] [125907-65-5] $C_{23}H_{34}N_2O_{14}$. A solution of the above protected glycoasparagine (0.23 g, 0.35 mmol) in MeOH (20 mL) was hydrogenated at 50 psi for 4 h with 10 % Pd/C catalyst (0.06 g). The reaction was filtered and concentrated to provide the glycosylasparagine acid (0.18 g, 0.32 mmol, 90%): MS (FAB) m/z 563.2108 ([M + H]$^+$, calcd 563.2088), 517, 507, 489, 463, 417, 331, 301, 169, 57.

L-Histidinamide, N-[2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^\alpha$-methyl-1-[(4-methylphenyl)sulfonyl]-, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] $C_{75}H_{105}N_{11}O_{22}S$.* A solution of the protected oligopeptide **29** (0.256 g, 0.25 mmol) and the above glycoasparagine (0.182 g, 0.32 mmol) were coupled under standard $(EtO)_2P(O)CN$ to provide, after silica flash chromatography, 0.246 g of the protected glycopeptide (0.16 mmol, 62%): TLC R_f 0.53 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1544.7 ([M + H]$^+$, calcd 1544.7), 1390.7, 1094.3, 331.1, 278.1, 169.0, 125.0.

L-Histidinamide, N-[β-D-mannopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N$^\alpha$-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] (10) $C_{60}H_{91}N_{11}O_{16}$.* The protected glycopeptide above (0.24 g) was dissolved in 3 M NH_3 in MeOH (9 mL). After 20 h the reaction was concentrated, and the product purified by silica flash chromatography to provide 0.12 g (0.10 mmol, 63 %) of **10**: TLC R_f 0.24 (6:4 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1222.677 ([M + H]$^+$, calcd 1222.672), 1206, 1122, 1088, 365, 289, 124, 109, 70, 57.

*Preparation of glycopeptide **11***

D-Glucopyranose, 2-(trifluoroacetylamino)-2-deoxy- [36875-26-0] $C_8H_{12}F_3NO_6$. To a solution of D-glucosamine hydrochloride (4.44 g, 20.5 mmol) in dry MeOH (50 mL) was added LiOH·H_2O (1.03 g, 24.5

mmol) and F_3COSEt (4 mL, 31.2 mmol). The reaction was stirred for 15 h at ambient temperature, at which time a second equal portion of F_3COSEt was added. After an additional 24 h the reaction was concentrated, and the residue dissolved in hot MeOH (200 mL). This solution was concentrated to 100 mL and then left at –20 °C for 72 h, at which time the unreacted glucosamine had precipitated as a white solid. The solution was separated from this precipitate by centrifugation, and concentrated to 18 mL with some precipitation of product. To this was added $CHCl_3$ (7 mL), which dissolved nearly all of this material, and the product purified by silica flash chromatography in 1:1 $CHCl_3$:MeOH, to provide a yellow oil after concentration. This oil hardened on the vacuum line to a hygroscopic solid (5.7 g, 107 % mass yield), and by NMR was an α,β-anomeric mixture: TLC R_f 0.82 (3:2 MeOH:$CHCl_3$); ^1H NMR (CD_3OD) *inter alia* δ 5.17 (J = 2.4 Hz), 4.73 (J = 8.2 Hz); ^{13}C NMR (CD_3OD) *inter alia* δ 96.86 (α), 96.09 (β).

β-D-Glucopyranose, 1,3,4,6-tetra-O-acetyl-2-(trifluoroacetylamino)-2-deoxy- [137766-83-7] $C_{16}H_{20}F_3NO_{10}$. The entire portion of the *N*-trifluoroacetylglucosamine was dissolved, over 30 min with stirring, in dry pyridine (35 mL) at 0 °C, and acetic anhydride (22 mL) added slowly. After stirring overnight at ambient temperature, the reaction was poured into cold aq. citric acid (50 g in 500 mL). The mixture was extracted (2 × 250 mL EtOAc). The combined organics were back-extracted with small portions of aq. HCl, water, aq. $NaHCO_3$, and water; and then dried and concentrated to an oil. The product (5.50 g) was obtained as a solid by Et_2O trituration. The material was an anomeric mixture (α anomer, major), from which 0.85 g (1.92 mmol, 9 %) of the β anomer was obtained by silica flash chromatography: TLC (anomeric mixture) R_f 0.55, 0.66 (9:1 CH_2Cl_2:acetone).

β-D-Glucopyranosyl azide, 3,4,6-tri-O-acetyl-2-(trifluoroacetylamino)-2-deoxy- $C_{14}H_{17}F_3N_4O_8$. A solution of the above protected saccharide (0.85 g, 1.92 mmol) in 20 mL CH_2Cl_2 was treated at ambient temperature under Ar with 0.31 mL Me_3SiN_3, and then dropwise with 1.95 mL of 1 M $SnCl_4$ in CH_2Cl_2. The reaction was stirred for 2 h and then quenched with 150 mL ice water. This mixture was extracted with CH_2Cl_2(3 × 150 mL) and the combined organics washed with satd aq. $NaHCO_3$ and water, and evaporated, to yield 0.75 g (1.76 mmol, 91%) of the azide: TLC R_f 0.09 (95:5 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ 6.79 (d, 1H, J = 8.8 Hz), 5.29 (1:2:1 t, 1H, J = 9.5 Hz), 5.13 (1:2:1 t, 1H, J = 9.6 Hz), 4.79 (d, 1H, J = 9.3 Hz), 4.30 (dd, 1H, J = 12.5, 4.8 Hz), 4.20 (dd, 1H, J = 12.5, 2.3 Hz), 4.00 (q, 1H, J = 9.5 Hz), 3.85 (ddd, 1H, ω = 19 Hz), 2.22 (s, 3H), 2.06 (s, 6H); MS (FAB) m/z 427.1074 ([M + H]$^+$, calcd 427.1077).

β-D-Glucopyranosylamine, 3,4,6-tri-O-acetyl-2-(trifluoroacetylamino)-2-deoxy- $C_{14}H_{19}F_3N_2O_8$. A solution of the above azide (1.0 g, 2.35 mmol) in abs. EtOH (130 mL) was hydrogenated at atmospheric pressure for 2.5 h, using 0.54 g 5 % Pd/$CaCO_3$ as catalyst. The catalyst was removed by filtration, and the solvent removed to provide 0.88 g (2.20 mmol, 93 %) of the amine: TLC R_f 0.31 (4:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ 6.67 (d, 1H, J = 9 Hz), 5.12 (m, 2H), 4.22 (dd, 1H, J = 12.2, 4.7 Hz) 4.16 (dd, 1H, J = 13, 2.6 Hz), 3.98 (q, 1H, J = 9.6 Hz), 3.70 (m, 1H, ω = 18 Hz), 2.11 (s, 3H), 2.05 (s, 6H); MS (FAB) m/z 401.1151 ([M + H]$^+$, calcd 401.1172), 384, 281, 264, 222, 204, 192, 43.

L-Asparagine, N²-[(1,1-dimethylethoxy)carbonyl]-N-[3,4,6-tri-O-acetyl-2-(trifluoroacetylamino)-2-deoxy-β-D-glucopyranosyl]-1-(phenylmethyl) ester $C_{30}H_{38}F_3N_3O_{13}$. A solution of the above amine (0.44 g, 1.1 mmol) and *N*-[(1,1-dimethylethoxy)carbonyl]-L-aspartic acid-1-(phenylmethyl) ester (0.446 g, 1.38 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide after flash chromatography 0.31 g (0.44 mmol, 40 %) of the glycosylasparagine ester: TLC R_f 0.34 (9:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ 7.34 (m, 5H), 6.68 (d, 1H, J = 8.5 Hz), 5.64 (d, 1H), 5.15 (s, 2H), 5.21 (1:2:1 t, 1H, J = 9 Hz), 5.09 (m, 3H), 4.55 (m, 1H), 4.30 (m, 1H), 4.06 (m, 2H), 2.79 (m, 1H), 2.9–2.6 (m, 3H), 2.08 (s, 3H), 2.06 (s, 6H), 1.41 (s, 9H); MS (FAB) m/z 706.2442 ([M + H]$^+$, calcd 706.2435), 606, 564, 516, 470, 264, 222, 204, 192.

L-Asparagine, N²-[(1,1-dimethylethoxy)carbonyl]-N-[3,4,6-tri-O-acetyl-2-(trifluoroacetylamino)-2-deoxy-β-D-glucopyranosyl]- $C_{23}H_{32}F_3N_3O_{13}$. The entire portion of the above protected glycoasparagine was dissolved in 29 mL MeOH, and hydrogenated at 30 psi for 4 h with 0.085 g 10 % Pd/C catalyst. Filtration and evaporation of the solvent provided 0.27 g (0.43 mmol, 97 %) of the glycosylasparagine acid: TLC R_f 0.22 (4:1 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 638.1756 ([M + Na]$^+$, calcd 638.1785), 615, 560, 516, 470, 264, 222, 204, 192, 179, 133, 92, 70, 57, 43.

L-Histidinamide,, N-[3,4,6-tri-O-acetyl-2-(trifluoroacetylamino)-2-deoxy-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl-1-[(4-methylphenyl)sulfonyl]-[1S[1R,2R*,4R*(1R*,2R*)]]* $C_{75}H_{103}F_3N_{12}O_{20}S$. A solution of the protected oligopeptide **28** (0.343 g, 0.35 mmol) and the above glycoasparagine (0.27 g, 0.43 mmol) were coupled under standard $(EtO)_2P(O)CN$ to provide after flash chromatography 0.57 mmol (0.36 mmol, 83 %) of the protected glycopeptide as a pale yellow solid: TLC R_f 0.41 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1582 ([M + H]$^+$, calcd 1582), 1428, 278, 222, 155, 124.

L-Histidinamide, N-[3,4,6-tri-O-acetyl-2-(trifluoroacetylamino)-2-deoxy-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl-1-[(4-methylphenyl)sulfonyl],N-oxide[1S[1R,2R*,4R*(1R*,2R*)]]* $C_{75}H_{103}F_3N_{12}O_{21}S$. The above protected glycopeptide (0.57 g, 0.35 mmol) in $CHCl_3$ (15 mL)

was reacted for 2 h with 3-$ClPhCO_3H$ (0.244 g) at ambient temperature under Ar. The reaction was quenched by partitioning between cold aq. Na_2SO_3 (150 mL) and $CHCl_3$ (150 mL). The aqueous layer was extracted with three additional portions of $CHCl_3$, dried, and concentrated to provide 0.57 g (0.36 mmol, 97 %) of the protected glycopeptide *N*-oxide: TLC R_f 0.37 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1598 ($[M + H]^+$, calcd 1598).

L-Histidinamide, N-[2-amino-2-deoxy-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]]* **(11)** *$C_{60}H_{92}N_{12}O_{15}$.* The above protected glycopeptide (0.56 g) was dissolved in MeOH (6 mL), and reacted at ambient temperature under Ar with 4 M NH_3 in MeOH (10 mL). After 40 h the reaction was concentrated, and the product purified by silica flash chromatography to provide 0.193 g (0.16 mmol, 45 %) of **11**: TLC R_f 0.19 (7:3 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1221.692 ($[M + H]^+$, calcd 1221.688), 1205, 365, 256, 239, 211, 196, 162, 124, 109, 86, 70, 57.

Preparation of glycopeptide 12

α-D-Galactopyranosylchloride, 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy- [41355-44-6] $C_{14}H_{20}ClNO_8$. A solution of 1,3,4,6-tetra-*O*-acetyl-2-(acetylamino)-2-deoxy-galactose (6.3 g) in CH_3COCl (15 mL) was stirred for 42 h at ambient temperature, cooled, and diluted with cold CH_2Cl_2 (150 mL). The reaction was quenched with ice water (200 g), and the organic layer immediately separated and washed thoroughly with 200 mL satd aq. $NaHCO_3$. The dark-colored organic layer was dried ($MgSO_4$) and concentrated to provide the tetraacetylgalactosylchloride.

β-D-Galactopyranosyl azide, 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy- $C_{14}H_{20}N_4O_8$. A suspension of the glycosylchloride (7.2 g) and NaN_3 (3.5 g) in formamide (75 mL) was stirred for 3 h under Ar at 85 °C, and then cooled and diluted with ice water (300 g). The suspension was extracted with $CHCl_3$ (3 × 250 mL), dried, and concentrated to a dark foam. The product was purified by silica flash chromatography to provide 3.60 g (9.67 mmol, 42 %) of the azide: TLC R_f 0.21 (2:1 EtOAc:hexanes); ^1H NMR ($CDCl_3$) : δ 5.68 (d, 1H), 5.36–5.17 (m, 2H), 4.77 (d, 1H), 4.18–3.93 (m, 4H), 2.15 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H); MS (FAB) m/z 373.1350 ($[M + H]^+$, calcd 373.1359), 361, 347, 318, 288, 270, 210, 168, 150, 126, 114, 43.

β-D-Galactopyranosylamine, 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy- $C_{14}H_{22}N_2O_8$. A solution of the azide (3.60 g, 9.67 mmol) in abs. EtOH (300 mL) was hydrogenated at atmospheric pressure for 2.5 h, using 1.54 g 5 % $Pd/CaCO_3$ as catalyst. The catalyst was removed by filtration, and the solvent removed to provide 3.23 g (9.33 mmol, 96 %) of the amine: TLC R_f 0.24 (4:1 EtOAc:EtOH); ^1H NMR ($CDCl_3$) : δ 5.62 (d, 1H), 5.31 (d, 1H), 5.02–4.93 (m, 1H), 4.30–3.92 (m, 6H), 3.85 (t, 1H), 2.15 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H); MS (FAB) m/z 347 ($[M + H]^+$, calcd 347), 330, 287, 227, 210, 168, 150, 138, 126, 108, 98, 43.

L-Asparagine, N²-[(1,1-dimethylethoxy)carbonyl]-N-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]-1-(phenylmethyl) ester $C_{30}H_{41}N_3O_{13}$. A solution of the above amine (0.50 g, 1.45 mmol) and L-aspartic acid, *N*-[1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester (0.59 g, 1.83 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide after flash chromatography 0.50 g (0.76 mmol, 52 %) of the glycosylasparagine ester: TLC R_f 0.23 (4:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ 7.35 (s, 5H), 7.12 (d, 1H), 5.76 (d, 1H), 5.61 (d, 1H), 5.33–4.87 (m, 4H), 4.6 (m, 1H), 4.38–4.03 (m, 4H), 3.96 (t, 1H), 2.94–2.61 (m, 2H), 2.15 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H), 1.42 (s, 9H); MS (FAB) m/z 652.2738 ($[M + H]^+$, calcd 652.2717), 596, 554, 458, 330, 223, 210, 168, 150, 126, 108, 91, 57, 43.

L-Asparagine, N²-[(1,1-dimethylethoxy)carbonyl]-N-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl] $C_{23}H_{35}N_3O_{13}$. A suspension of the above protected glycoasparagine (0.49 g, 0.75 mmol) in 50 mL MeOH was hydrogenated at 30 psi for 4 h with 0.16 g 10 % Pd/C catalyst. The reaction was filtered and concentrated to provide 0.38 g (0.68 mmol, 90 %) of the glycosylasparagine acid: TLC R_f 0.17 (4:1 CH_2Cl_2:4 M NH_3 in MeOH); ^1H NMR ($CDCl_3$) : δ 7.06 (m, 1H), 5.38 (m, 1H), 5.18–5.12 (m, 3H), 4.56–4.47 (m, 1H), 4.41–4.18 (m, 1H), 4.17–4.02 (m, 4H), 2.98–2.61 (m, 2H), 2.16 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.44 (s, 9H); MS (FAB) m/z 562.2243 ($[M + H]^+$, calcd 562.2248), 506, 462, 368, 330, 210, 177, 168, 150, 126, 108, 57, 43.

L-Histidinamide, N-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl-1-[(4-methylphenyl)sulfonyl]-, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] $C_{75}H_{106}N_{12}O_{21}S$.* A solution of the protected oligopeptide **29** (0.356 g, 0.356 mmol) and the above glycoasparagine acid (0.25 g, 0.44 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide, after silica flash chromatography, 0.29 g of the protected glycopeptide (0.19 mmol, 53 %): TLC R_f 0.49 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1544 ($[M + H]^+$, calcd 1544).

L-Histidinamide, N-[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]-N²-[(1,1-dimethylethoxy) carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] (12) $C_{62}H_{94}N_{12}O_{16}$.* The above protected glycopeptide (0.26 g) was dissolved in MeOH (6 mL), and reacted at ambient temperature under Ar

with 7 M NH_3 in MeOH (12 mL). After 18 h the reaction was concentrated, and the product purified by flash silica chromatography to provide 0.189 g (0.15 mmol, 89 %) of **12**: TLC R_f 0.11 (4:1 CH_2Cl_2:7 M NH_3 in MeOH); MS (FAB) m/z 1263.703 ($[M + H]^+$, calcd 1263.699), 1247, 1163, 602, 365, 204, 124, 109, 86, 70, 57.

Preparation of glycopeptide ***13***

β-D-Galactopyranosyl azide, 2,3,4,6-tetra-O-acetyl-[13992-26-2] $C_{14}H_{19}N_3O_9$. A solution of pentaacetyl-β-D-galactose (4.81 g, 12.3 mmol) in 100 mL CH_2Cl_2 was treated at ambient temperature under Ar with $(CH_3)_3SiN_3$ (1.99 mL) followed by the dropwise addition of 12.5 mL of 1 M $SnCl_4$ in CH_2Cl_2. After 2 h, the reaction was partitioned between 300 mL ice water and 300 mL CH_2Cl_2. The aqueous mixture was extracted with two additional portions of CH_2Cl_2, and the combined organics washed with aq. $NaHCO_3$; dried; and concentrated to provide 4.6 g (12.3 mmol, 100 %) of the azide: TLC R_f 0.63 (9:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ 5.42 (dd, 1H, J = 4, 2 Hz), 5.17 (dd, 1H, J = 10.4, 8.7 Hz), 5.03 (dd, 1H, J = 10.4, 3.3 Hz), 4.60 (d, 1H, J = 8.7 Hz), 4.20–4.15 (m, 2H), 4.02 (1:2:1 t, 1H, J = 7.2 Hz), 2.18 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H); MS (FAB) m/z 374.1193 ($[M + H]^+$, calcd 374.1199).

β-D-Galactopyranosylamine, 2,3,4,6-tetra-O-acetyl-[58484-22-3] $C_{14}H_{21}NO_9$. The above azide (4.7 g) in abs. EtOH (400 mL) was hydrogenated at atmospheric pressure for 2.5 h, using 1.8 g 5 % Pd:$CaCO_3$ as catalyst. The catalyst was removed by filtration, and the solvent removed to provide 4.3 g (2.38 mmol, 98 %) of the amine: TLC R_f 0.56 (4:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ 5.41 (d, 1H), 5.03 (m, 2H), 4.14 (m, 3H), 3.91 (t, 1H), 2.16 (s, 3H), 2.1 (m, 2H), 2.09 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H); MS (FAB) m/z 348.1294 ($[M + H]^+$, calcd 348.1294).

L-Asparagine, N^2-[(1,1-dimethylethoxy)carbonyl]-N-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl]-1-(phenylmethyl) ester $C_{30}H_{40}N_2O_{14}$. The above amine (0.50 g, 1.44 mmol) and *N*-[1,1-dimethylethoxy)carbonyl]-L-aspartic acid 1-(phenylmethyl) ester (0.582 g, 1.80 mmol) were coupled under standard $(EtO)_2$P(O)CN conditions to provide, after flash silica chromatography 0.41 g (0.63 mmol, 44 %) of the glycosylasparagine ester: TLC R_f 0.31 (9:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ 7.34 (m, 5H), 6.41 (d, 1H), 5.43 (d, 1H), 5.19–5.09 (m, 6H), 4.60 (m, 1H), 4.10 (m, 4H), 2.8 (m, 1H), 2.14 (s, 3H), 2.05 (s, 3H), 2.00 (s, 6H), 1.42 (s, 9H); MS (FAB) m/z 653.2556 ($[M + H]^+$, calcd 653.2558), 597, 553, 511, 331, 289, 223, 169, 154.

L-Asparagine, N^2-[(1,1-dimethylethoxy)carbonyl]-N-[2,3,4,6-tetra-O-acetyl-2-β-D-galactopyranosyl]- $C_{23}H_{34}N_2O_{14}$. The entire portion of the above protected glycoasparagine was hydrogenated in 39 mL MeOH at 30 psi for 4 h with 0.135 g 10 % Pd/C catalyst. Removal of the catalyst by filtration, and evaporation of the solvent, provided 0.32 g (0.58 mmol, 92 %) of the glycosylasparagine acid: TLC R_f 0.21 (4:1 CH_2Cl_2:5 M NH_3 in MeOH); ^1H NMR ($CDCl_3$) : δ 6.79 (d, 1H, J = 9 Hz), 5.93 (d, 1H), 5.45–5.35 (m, 5H), 5.28 (1:2:1 t, 1H, J = 9 Hz), 4.2 (m, 3H), 2.7 (m, 2H), 2.15 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.44 (s, 9H); MS (FAB) m/z 563.2097 ($[M + Na]^+$, calcd 563.2088), 507, 485, 463, 421, 331, 289, 169, 154, 127, 109, 57, 43.

L-Histidinamide, N-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino] carbonyl]hexyl]-N^α-methyl-1-[(4-methylphenyl)sulfonyl], N-oxide [1S[1R,2R*,4R*(1R*,2R*)]]* $C_{75}H_{105}N_{11}O_{22}S$. The above glycoasparagine (0.25 g, 0.44 mmol) and the protected peptide **29** in CH_2Cl_2 (15 mL) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide after silica flash chromatography 0.345 g (0.22 mmol, 50 %) of the protected glycopeptide: TLC R_f 0.63 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1544.6 ($[M + H]^+$, calcd 1544.7), 1390.6, 331.1, 278.1, 169.0, 125.0.

L-Histidinamide, N-[β-D-galactopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-N^α-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] (13)* $C_{60}H_{91}N_{11}O_{16}$. The entire above protected glycopeptide (0.34 g, 0.22 mmol) was dissolved in 6 mL MeOH, and reacted at ambient temperature under Ar with 12 mL 6 M NH_3 in MeOH. After 16 h the reaction was concentrated, and the residue purified by silica flash chromatography to provide 0.24 g (0.20 mmol, 88 %) of the glycopeptide **13**: TLC R_f 0.21 (7:3 CH_2Cl_2:6 M NH_3 in MeOH); MS (FAB) m/z 1222.7 ($[M + H]^+$, calcd 1222.7).

Preparation of glycopeptide ***14***

β-L-Galactopyranosyl azide, 6-deoxy-2,3,4-tri-O-acetyl-[95581-07-0] $C_{12}H_{17}N_3O_7$. A solution of triacetylfucosyl chloride (2.96 g, 9.58 mmol) in formamide (30 mL) was treated with NaN_3 for 3 h at 85 °C under N_2. The reaction was cooled, diluted with water, and extracted with $CHCl_3$. The combined organics were dried and concentrated, and the residue was purified by silica flash chromatography to yield 2.0 g (6.34 mmol, 66 %) of the azide: TLC R_f 0.53 (1:1 EtOAc:hexanes); ^1H NMR : δ 5.27 (d, 1H, J = 3.3 Hz), 5.14 (dd, 1H, J = 10.3, 8.6 Hz), 5.03 (dd, 1H, J = 10.3, 3.3 Hz), 4.59 (d, 1H, J = 8.6 Hz), 3.91 (q, 1H, J = 6.4 Hz), 2.20 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H), 1.26 (d, 3H, J = 6.4 Hz); MS (FAB) m/z 316.1162 ($[M + H]^+$, calcd 316.1145), 288, 273, 256, 231, 213, 201, 171, 153, 137, 111, 99, 83.

β-L-Galactopyranosylamine, 6-deoxy-2,3,4-tri-O-acetyl- $C_{12}H_{19}NO_7$. A suspension of the azide (2.0 g, 6.34 mmol) was hydrogenated at atmospheric pressure for 2.5 h with 0.85 g 5% Pd:$CaCO_3$ as catalyst. The reaction was filtered and concentrated to provide 1.80 g (6.22 mmol, 96 %) of the glycosylamine: TLC R_f 0.14

(9:1 CH_2Cl_2:acetone); ^1H NMR : δ 5.26 (d, 1H, J = 3.0 Hz), 5.10–4.95 (m, 2H), 4.13 (d, 1H, J = 8.1 Hz), 3.81 (q, 1H, J = 6.4 Hz), 2.18 (s, 3H), 2.09 (s, 3H), 2.0 (m, 2H), 1.98 (s, 3H), 1.19 (d, 3H, J = 6.4 Hz); MS (FAB) m/z 290 ($[M + H]^+$, calcd 290), 273, 213, 171, 153, 129, 111, 83, 43.

L-Asparagine, N²-[(1,1-dimethylethoxy)carbonyl]-N-[6-deoxy-2,3,4-tri-O-acetyl-β-L-galactopyranosyl]-1-(phenylmethyl) ester $C_{28}H_{38}N_2O_{12}$. The above glycosylamine (1.25 g, 4.32 mmol) and L-aspartic acid, *N*-[1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester (1.75 g, 5.40 mmol) in CH_2Cl_2 (60 mL)were coupled under standard $(EtO)_2P(O)CN$ conditions, to provide after silica flash chromatography 1.17 g (1.97 mmol, 45 %) of the protected glycosylasparagine ester: TLC R_f 0.32 (9:1 CH_2Cl_2:acetone); ^1H NMR : δ 7.34 (m, 5H), 6.33 (m, 1H), 5.54 (m, 1H), 5.28–5.03 (m, 6H), 4.54 (m, 1H), 3.88 (q, 1H), 3.03–2.72 (dq, 2H), 2.17 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.42 (s, 9H), 1.19 (d, 3H); MS (FAB) m/z 595.2524 ($[M + H]^+$, calcd 595.2503), 539, 495, 396, 273, 213, 171, 153, 127, 111, 91, 57, 43.

L-Asparagine, N²-[(1,1-dimethylethoxy)carbonyl]-N-[6-deoxy-2,3,4-tri-O-acetyl-β-L-galactopyranosyl]- $C_{21}H_{32}N_2O_{12}$. A suspension of the above protected glycoasparagine (0.67 g) was hydrogenated in MeOH (80 mL) at 30 psi for 4 h with 0.15 g Pd/C as catalyst. The reaction was filtered and concentrated to give 0.54 g (1.07 mmol, 95 %) of the glycosylasparagine acid: ^1H NMR : δ 6.98 (m, 1H), 5.76 (m, 1H), 5.31–5.12 (m, 6H), 4.50 (m, 1H), 4.03 (m, 1H), 3.96 (t, 1H), 3.06 (m, 1H), 3.03–2.62 (dq, 2H), 2.18 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.45 (s, 9H), 1.19 (d, 3H); MS (FAB) m/z 505.2033 ($[M + H]^+$, calcd 505.2033), 449, 405, 273, 213, 171, 153, 127, 111, 83, 57, 43.

L-Histidinamide, N-[6-deoxy-2,3,4-tri-O-acetyl-β-L-galactopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl-1-[(4-methylphenyl)sulfonyl] [1S-[1R,2R*,4R*(1R*,2R*)]]* $C_{73}H_{103}N_{11}O_{19}S$. The protected peptide **28** (0.512 g, 0.52 mmol) and the above glycosylasparagine acid (0.33 g, 0.654 mmol) in CH_2Cl_2 (13 mL) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide after silica flash chromatography 0.51 g (0.35 mmol, 67 %) of the protected glycopeptide: TLC R_f 0.26 (95:5 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1471 ($[M + H]^+$, calcd 1471).

L-Histidinamide, N-[6-deoxy-2,3,4-tri-O-acetyl-β-L-galactopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl-1-[(4-methylphenyl)sulfonyl], N-oxide [1S[1R,2R*,4R*(1R*,2R*)]]* $C_{73}H_{103}N_{11}O_{20}S$. The above protected glycopeptide (0.40 g, 0.27 mmol) and 3-$ClPhCO_3H$ (0.181 g) in $CHCl_3$ (11 mL) were stirred for 2 h, diluted with cold aq. 10 % $NaSO_3$, and extracted with $CHCl_3$ (4 × 150 mL). The combined organics were dried and concentrated to provide 0.47 g (0.27 mmol, quantitative) of the protected glycopeptide N-oxide: TLC R_f 0.32 (9:1 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1487 ($[M + H]^+$, calcd 1487).

L-Histidinamide, N-[6-deoxy-β-L-galactopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] (14)* $C_{60}H_{91}N_{11}O_{15}$. A solution of the protected glycopeptide (0.47 g, 0.27 mmol) in MeOH (6 mL) was treated at ambient temperature under N_2 with 4 M NH_3 in MeOH (6 mL). After 18 h the reaction was concentrated, and the residue purified by silica flash chromatography to give 0.30 g (0.25 mmol, 93 %) of the glycopeptide **14**: TLC R_f 0.13 (4:1 CH_2Cl_2 : 4 M NH_3 in MeOH); MS (FAB) m/z 1206.677 ($[M + H]^+$, calcd 1206.677), 1190, 656, 602, 365, 222, 212, 196, 124, 109, 86, 70, 57.

Preparation of glycopeptide 15

β-D-Glucopyranosyl azide, 4-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl]-2,3,6-tri-O-acetyl [33012-49-6] $C_{26}H_{35}N_3O_{17}$. To a suspension of acetobromomaltose (9.9 g, 14.2 mmol) in dry formamide (25 mL) was added NaN_3 (1.49 g, 22.0 mmol), and the reaction was stirred for 20 h at ambient temperature. It was diluted with $CHCl_3$ (100 mL), and extracted with four portions of water (100 mL apiece), which are discarded. The solution was dried and concentrated to a brown oil. The residue was purified by silica chromatography (40:1 $CHCl_3$: MeOH) to give the azide as a light brown-colored foamy residue: TLC R_f 0.72 (50:1 $CHCl_3$:4 M NH_3 in MeOH); IR 1230, 2120 cm^{-1}; ^1H NMR ($CDCl_3$) *inter alia* : δ 4.72 (d, J = 8.8 Hz), 5.42 (d, J = 4.0 Hz).

β-D-Glucopyranosylamine, 4-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl]-2,3,6-tri-O-acetyl- $C_{26}H_{37}NO_{17}$. The entire portion of the glycosylazide was dissolved in EtOAc (15 mL) and hydrogenated over 5 % Pd:$CaCO_3$ (0.98 g, pre-reduced in 85 mL abs. EtOH) at ambient temperature and pressure. After 3 h the reaction was filtered and concentrated, and the residue purified by silica flash chromatography to give the amine as an oil, which foams and hardens on the vacuum line into a fluffy, granular white solid (3.66 g, 5.7 mmol). The overall yield from acetobromomaltose is 40 %. TLC R_f 0.44 (50:1 $CHCl_3$:4 M NH_3 in MeOH); ^1H NMR ($CDCl_3$) : δ *inter alia* 5.41 (d, 1H, J = 4.0 Hz), 5.33 (m, 2H), 5.06 (t, 1H, J = 9.8 Hz), 4.86 (dd, 1H, J = 10.5, 4.0 Hz), 4.67 (t, 1H, J = 9.3 Hz), 4.46 (dd, 1H, J = 12.0, 2.0 Hz), 4.22 (m, 3H), 4.00 (m, 3H), 3.68 (m, 1H); ^{13}C NMR ($CDCl_3$) : δ *inter alia* 95.49, 84.42, 75.67, 73.04 (2C), 72.91, 69.98, 69.32, 68.47, 68.01, 63.17, 61.48; MS (EI) m/z (rel intensity) 576 ($(M - CH_3CO_2)^+$, 10), 531 (6.8), 331 (anomeric fragmentation, 45), 169 (100); (CI, NH_3) 636 ($[M + H]^+$, 76), 618 (48), 366 ([tetraacetyl glucose + H]$^+$, 100).

L-Asparagine, N-[2,3,6-tri-O-acetyl-4-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl]-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester $C_{42}H_{56}N_2O_{22}$. To a solution of the glycosylamine (1.24 g, 2.00 mmol) and L-aspartic acid-*N*-[1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester (0.65 g, 2.00 mmol) in CH_2Cl_2 (5 mL) was added 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (0.68 g, 2.48 mmol). The solution was stirred at ambient temperature for 5 d, at which time it was diluted with CH_2Cl_2; washed with dilute citric acid and brine; dried; and concentrated. The residue was purified by silica chromatography to provide 1.08 g (1.14 mmol, 57 %) of the protected glycosylasparagine ester: mp 97–99 °C; TLC R_f 0.36 (40:1 $CHCl_3$:MeOH); ^1H NMR ($CDCl_3$) : δ *inter alia* 6.27 (d, 1H, *J* = 9.3 Hz), 5.72 (d, 1H, *J* = 8.8 Hz), 5.39 (d, 1H, *J* = 3 Hz), 5.34 (d, 1H, *J* = 10.6 Hz), 5.22 (1:2:1 t, 1H, *J* = 9.3 Hz; collapsed with D_2O exchange of the δ 6.27 amide H to a d, *J* = 9.4 Hz), 5.20 (s, 2H), 5.07 (1:2:1 t, 1H, *J* = 9.8 Hz), 4.86 (dd, 1H, *J* = 10.5, 4 Hz), 4.73 (1:2:1 t, 1H, *J* = 9.6 Hz), 4.57 (m, 1H, ω = 28 Hz), 4.42 (dd, 1H, *J* = 12.5, 2 Hz), 4.26–4.18 (m, 2H), 4.08–3.90 (m, 3H), 3.76 (br d, 1H, Δυ approx 9 Hz), 2.84 (br d dd, 1H, *J* = 16,4 Hz), 2.70 (dd, 1H, *J* = 16,4 Hz); ^{13}C NMR ($CDCl_3$) : δ *inter alia* C:155.26, 135.10, 79.54; CH: 128.29 (2C), 128.01, 127.63 (2C), 95.34, 77.10, 74.98, 73.66, 72.50, 70.90, 69.63, 68.93, 68.19, 67.60, 49.82; CH_2: 66.82, 62.53, 61.10, 37.24; CH_3: 27.90 (3 C); MS (FAB) m/z (rel intensity) 941 ($[M]^+$, 0.6), 841 ($[M - Boc + 2H]^+$, 16), 331 (anomeric fragmentation, 16), 169 (100).

L-Asparagine, N-[2,3,6-tri-O-acetyl-4-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl]-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]- $C_{35}H_{50}N_2O_{22}$. A suspension of the protected glycosyl asparagine ester (0.776 g, 0.82 mmol) and 10 % Pd:C (0.216 g) in MeOH (75 mL) was hydrogenated at 30 psi for 3.5 h. The reaction was filtered and concentrated to provide 0.52 g (0.61 mmol, 75 %) of the glycosylasparagine acid: TLC R_f 0.31 (4:1 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 851.2927 ($[M + H]^+$; calcd 851.2933), 873, 751, 331, 271, 229, 211.

L-Histidinamide, N-[2,3,6-tri-O-acetyl-4-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl]-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl-1-[(4-methylphenyl)sulfonyl]-N-oxide [1S[1R,2R*,4R*(1R*,2R*)]]* $C_{87}H_{121}N_{11}O_{30}S$. The protected peptide **29** (0.31 g, 0.31 mmol) and the above glycosylasparagine acid (0.33 g, 0.39 mmol) were coupled under standard $(EtO)_2POCN$ conditions to provide after silica chromatography 0.426 g (0.23 mmol, 74 %) of the protected glycopeptide: TLC R_f 0.53 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1832.7 ($[M + H]^+$; calcd 1832.8), 1678.8, 1382.5, 519.3, 371.3, 331.1, 278.1, 211.1.

L-Histidinamide, N-[4-O-(α-D-glucopyranosyl)-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] (15)* $C_{66}H_{101}N_{11}O_{21}$. A solution of the protected glycopeptide (0.42 g, 0.23 mmol) in MeOH (6 mL) at ambient temperature under Ar was treated with 5 M NH_3 in MeOH (15 mL) for 16 h. The product was purified by silica chromatography to give 0.244 g (0.176 mmol, 77 %) of the glycopeptide **15**: TLC R_f 0.13 (6:4 $CHCl_3$:5 M NH_3 in MeOH); MS (FAB) m/z (1384.7 $[M + H]^+$; calcd 1384.7).

Preparation of glycopeptide 16

β-D-Glucopyranose, 4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-1,2,3,6-tetra-O-acetyl [3616-19-1] $C_{28}H_{38}O_{19}$. A suspension of cellobiose (5.0 g, 14.6 mmol) and anhydrous NaOAc powder (4.0 g) in $(CH_3CO)_2O$ (50 mL) was heated in a 110 °C oil bath for 4.5 h under Ar. The suspension was filtered and concentrated to give a quantitative yield (9.95 g) of the peracetyl disaccharide: TLC R_f 0.33 (9:1 CH_2Cl_2:acetone).

β-D-Glucopyranosyl azide, 4-O-[2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl]-2,3,6-tri-O-acetyl-[33012-50-9] $C_{26}H_{35}N_3O_{17}$. The above acetylated disaccharide (2.0 g, 2.95 mmol) in CH_2Cl_2 (40 mL) at ambient temperature under Ar was treated with Me_3SiN_3 (0.48 mL), followed by the dropwise addition of 1 M $SnCl_4$ in CH_2Cl_2 (3.0 mL). After 20 h the reaction was poured into ice water (300 g) and extracted with CH_2Cl_2 (3 × 300 mL). The combined organics were washed with 300 mL of satd $NaHCO_3$, dried, filtered, and concentrated to give 1.8 g (2.72 mmol, 90 %) of the azide: TLC R_f 0.41 (9:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ *inter alia* 5.70 (1:2:1 t, 1H, *J* = 8.5 Hz), 5.13 (d, 1H, *J* = 9.5 Hz), 5.07 (1:2:1 t, 1H, *J* = 9.5 Hz), 4.93 (1:2:1 t, 1H, *J* = 8.2 Hz), 4.87 (1:2:1 t, 1H, *J* = 8.8 Hz), 4.62 (d, 1H, *J* = 8.8 Hz), 4.55–4.45 (m, 2H), 4.38 (dd, 1H, *J* = 12.5, 3.5 Hz), 4.11 (dd, 1H, *J* = 12.5, 4.5 Hz), 4.05 (dd, 1H, *J* = 13.5, 2 Hz), 3.80 (1:2:1 t, 1H, *J* = 9.0 Hz), 3.67 (m, 1H, ω = 27 Hz); MS (FAB) m/z 662 ($[M + H]^+$; calcd 662), 634, 619, 331, 289, 271, 229, 211, 169, 139, 127, 109, 97, 81.

β-D-Glucopyranosylamine, 4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-2,3,6-tri-O-acetyl- $C_{26}H_{37}NO_{17}$. The above azide (1.8 g, 2.72 mmol) in MeOH (300 mL) was hydrogenated at ambient temperature and pressure with 5 % Pd:$CaCO_3$ (0.76 g). The suspension was filtered and concentrated to provide 1.72 g (2.70 mmol, 95 %) of the disaccharide amine: TLC R_f 0.25 (4:1 CH_2Cl_2:acetone); MS (FAB) m/z 636 ($[M + H]^+$; calcd 636), 619, 594, 576, 559, 331, 169, 139, 127, 109, 97, 43.

L-Asparagine, N-[2,3,6-tri-O-acetyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-β-D-glucopyranosyl-N²-[(1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester $C_{42}H_{56}N_2O_{22}$. The above amine (0.74 g, 1.16 mmol) and *N*-[1,1-dimethylethoxy)carbonyl]-L-aspartic acid-1-(phenylmethyl) ester (0.469 g, 1.45 mmol) were coupled under standard $(EtO)_2P(O)CN$

conditions to provide after silica chromatography 0.33 g (0.35 mmol, 30 %) of the glycosylasparagine ester: TLC R_f 0.41 (4:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ *inter alia* 6.30 (d, 1H, J = 9.2 Hz), 5.25 (1:2:1 t, 1H, J = 9.2 Hz); MS (FAB) m/z 941.3389 ([M + H]$^+$; calcd 941.3403), 841, 537, 331, 271, 229, 211, 169, 154, 139, 127, 109, 91.

L-Asparagine, N-[2,3,6-tri-O-acetyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-β-D-glucopyranosyl-N²-[(1,1-dimethylethoxy)carbonyl]- $C_{35}H_{50}N_2O_{22}$. A suspension of the above protected glycoasparagine ester (0.32 g, 0.34 mmol) and 10 % Pd:C (0.085 g) in MeOH (30 mL) was hydrogenated at 30 psi for 4 h. The reaction was filtered and concentrated to give 0.285 g (0.33 mmol, 98 %) of the glycosylasparagine acid: MS (FAB) m/z 851.2923 ([M + H]$^+$; calcd 851.2933), 873, 795, 751, 331, 271, 229, 169, 154, 139, 127, 109.

L-Histidinamide, N-[2,3,6-tri-O-acetyl-4-O-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nᵅ-methyl-1-[(4-methylphenyl)sulfonyl]-, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]]* $C_{87}H_{121}IN_{11}O_{30}S$. The protected peptide **29** (0.26 g, 0.26 mmol) and the above glycosylasparagine acid (0.279 g, 0.33 mmol) in CH_2Cl_2 (7 mL) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide after silica chromatography 0.308 g (0.17 mmol, 65 %) of the protected glycopeptide: TLC R_f 0.63 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1832.8 ([M + H]$^+$; calcd 1832.8).

L-Histidinamide, N-[4-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl]-N²-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]-carbonyl]hexyl]-Nᵅ-methyl, N-oxide [1S[1R,2R*,4R*(1R*,2R*)]] (16)* $C_{66}H_{101}N_{11}O_{21}$. The above protected glycopeptide (0.30 g, 0.16 mmol) in MeOH (6 mL) was treated at ambient temperature with 5 M NH_3 in MeOH (12 mL). The reaction was concentrated after 15 h, and the residue purified by silica chromatography to give 0.182 g (0.13 mmol, 82 %) of the glycopeptide **16**: TLC R_f 0.20 (6:4:0.4 CH_2Cl_2:MeOH:satd aq. NH_3); MS (FAB) m/z 1384.8 ([M + H]$^+$; calcd 1384.8).

Preparation of glycopeptide 17

β-D-Glucopyranose, 2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-1,3,6-tri-O-acetyl- [7284-18-6] $C_{28}H_{40}N_2O_{17}$. This was prepared by the acid digestion and acetylation of chitin (U. S. Biochemical micropulverized).[32] ^1H NMR ($CDCl_3$) : δ *inter alia* 6.10 (d, 1H, J = 3.61 Hz), 6.00 (br d, 1H, J = 9.22 Hz), 5.64 (br d, 1H, J = 9.07 Hz); ^{13}C NMR (75 MHz, CD_3SOCD_3) : δ *inter alia* CH: 100.35, 89.72, 75.20, 72.40, 70.58, 70.31, 70.17, 68.18, 53.63, 50.02; CH_2: 61.75, 61.58; MS (EI) m/z (rel intensity) 432 (0.8), 390 (0.8), 330 (12), 241 (17), 199 (20), 181 (19), 168 (20), 156 (32), 139 (39), 114 (100); CI (isobutane) 677 ([M + H], 95), 330 (anomeric fragmentation, 100).

β-D-Glucopyranosyl chloride, 2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-3,6-di-O-acetyl-[7531-49-9] $C_{26}H_{37}ClN_2O_{15}$. To a suspension of the above peracetylated disaccharide (4.1 g, 6.06 mmol) in acetyl chloride (41 mL) at –20 °C under Ar was bubbled over 45 min HCl gas, at which time the reaction had darkened and the solid had dissolved. The solution was warmed to ambient temperature. After 25 h it was concentrated, and the residue recrystallized from acetone: ether, to give 2.03 g (3.11 mmol, 51 % yield) of the chloride. The mother liquor provided upon concentration an additional 2.0 g, contaminated by a more polar impurity. mp 187–189 °C; TLC R_f 0.26 (2:1 CH_2Cl_2: acetone); MS (FAB) m/z 655 ([M + H]$^+$), 653 ([M + H]$^+$), 617, 557, 515, 330, 288, 228, 210, 168, 150.

β-D-Glucopyranosyl azide, 2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-3,6-di-O-acetyl- [29625-70-5] $C_{26}H_{37}N_5O_{15}$. A suspension of the above chloride (2.0 g, 3.06 mmol) and NaN_3 (1.06 g) in formamide (20 mL) was stirred under N_2 at 80 °C for 3 h. The reaction was poured into ice water (200 mL) and extracted with $CHCl_3$ (3 × 200 mL). The combined organics were dried, filtered, and concentrated to give 1.55 g (2.35 mmol, 76 %) of the azide as a pale yellow solid: TLC R_f 0.19 (2:1 CH_2Cl_2:acetone); MS (FAB) m/z 660.2370 ([M + H]$^+$; calcd 660.2364), 635, 617, 599, 585, 557, 330, 228, 210, 168, 150, 138.

β-D-Glucopyranosylamine, 2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-3,6-di-O-acetyl- [29673-51-6] $C_{26}H_{39}N_3O_{15}$. A suspension of the above azide (1.55 g, 2.35 mmol) and 5 % Pd:$CaCO_3$ (0.65 g) in abs EtOH (90 mL) and MeOH (210 mL) was hydrogenated at ambient pressure for 2.5 h. The reaction was filtered and concentrated to provide 1.48 g (2.33 mmol, 95 %) of the amine: TLC R_f 0.14 (3:1 EtOAc:EtOH); MS (FAB) m/z 634.2448 ([M + H]$^+$; calcd 634.2459), 617, 574, 330, 227, 210, 168, 150, 126, 108, 43.

L-Asparagine, N-[3,6-di-O-acetyl-2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-β-D-glucopyranosyl-N²-[(1,1-dimethylethoxy)carbonyl]-1-(phenylmethyl) ester $C_{42}H_{58}N_4O_{20}$. The above glycosylamine (0.65 g, 1.03 mmol) and *N*-[1,1-dimethylethoxy)carbonyl]-L-aspartic acid-1-(phenylmethyl) ester (0.419 g, 1.29 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions to provide after silica chromatography 0.426 g (0.45 mmol, 44 %) of the glycosylasparagine ester: TLC R_f 0.28 (1:1 CH_2Cl_2:acetone); MS (FAB) m/z 939.3700 ([M + H]$^+$; calcd 939.3722).

L-Asparagine, N-[3,6-di-O-acetyl-2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-

deoxy-β-D-glucopyranosyl]-β-D-glucopyranosyl-N^2-*[(1,1-dimethylethoxy)carbonyl]- [98304-17-7]* $C_{35}H_{52}N_4O_{20}$. A suspension of the glycosylasparagine acid (0.42 g, 0.45 mmol), NH_4HCO_2 (0.426 g, 6.75 mmol), and 5 % Pd/C (0.21 g) in dimethylformamide was stirred under Ar. After 20 h at ambient temperature the reaction was filtered and concentrated to give 0.35 g (0.41 mmol, 91 %) of the glycosylasparagine acid: TLC R_f 0.21 (7:3 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 849.3260 ([M + H]$^+$; calcd 849.3253), 793, 749, 371, 330, 288, 240, 210, 168, 150, 126, 108, 57, 43.

L-Histidinamide, N-*[3,6-di-O-acetyl-2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-β-D-glucopyranosyl]*-N^2-*[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl*-N-*[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]*-N$^\alpha$-*methyl-1-[(4-methylphenyl)sulfonyl]-,* N-*oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{87}H_{123}N_{13}O_{27}S$. The protected peptide **29** (0.353 g, 0.36 mmol) and the glycosylasparagine acid (0.42 g, 0.45 mmol) in CH_2Cl_2 (9 mL) and DMF (3 mL) at ambient temperature under Ar was treated with Et_3N (0.063 mL, 0.45 mmol) and $(EtO)_2POCN$ (0.077 mL, 0.504 mmol). After 18 h the reaction was concentrated, and the residue purified by silica chromatography to give 0.476 g (0.26 mmol, 73 %) of the protected glycopeptide: TLC R_f 0.43 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1814.8 ([M + H]$^+$; calcd 1814.8), 1797, 1774, 1714, 1660.

L-Histidinamide, N-*[3,6-di-O-acetyl-2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-β-D-glucopyranosyl]*-N^2-*[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl*-N-*[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]*-N$^\alpha$-*methyl-1-[(4-methylphenyl)sulfonyl]-,* N-*oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{87}H_{123}N_{13}O_{28}S$. The protected glycopeptide (0.474 g, 0.26 mmol) and 3-$ClPhCO_3H$ (0.181 g) in $CHCl_3$ (11 mL) were stirred at ambient temperature under Ar for 2 h. The reaction was poured into 150 mL cold 10 % Na_2SO_3 and extracted with $CHCl_3$ (4 × 150 mL). The combined organics were dried, filtered, and concentrated to give 0.48 g (0.26 mmol, 95 %) of the protected glycopeptide *N*-oxide as a yellow solid: TLC R_f 0.31 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1831 ([M + H]$^+$; calcd 1831).

L-Histidinamide, N-*[4-O-[(2-(acetylamino)-2-deoxy-β-D-glucopyranosyl)]-β-D-glucopyranosyl]*-N^2-*[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-prolyl-L-phenylalanyl*-N-*[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]*-N$^\alpha$ *methyl,* N-*oxide [1S[1R*,2R*,4R*(1R*,2R*)]] (17)* $C_{70}H_{107}N_{13}O_{21}$. The protected glycopeptide (0.48 g, 0.26 mmol) in MeOH (6 mL) was treated at ambient temperature under Ar with 4 M NH_3 in MeOH (10 mL). After 18 h the reaction was concentrated, and the product purified by silica chromatography to give 0.308 g (0.21 mmol, 82 %) of glycopeptide **17**: TLC R_f 0.16 (6:4 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1466.5 ([M + H]$^+$; calcd 1466.7), 460.1, 371.2, 307.0.

Preparation of glycopeptide ***18***

S-*1,3-Dioxolane-4-acetic acid, 2,2-dimethyl-5-oxo-[73991-95-4]* $C_7H_{10}O_5$. A suspension of pyridinium *para*-toluene sulfonate (0.55 g) and *S*-malic acid (2.5 g, 18.6 mmol) in 2,2-dimethoxypropane (9 mL) was stirred at ambient temperature.[33] Virtually all of the solid dissolved within 30 min. After 48 h the light yellow-colored solution was concentrated; taken up in EtOAc; and passed through a silica column. The light yellow band was collected and concentrated to give (as an oily solid) 3.1 g (17.8 mmol, 95 %) of the product: ^{1}H NMR ($CDCl_3$) : δ 11.28 (br s, 1H), 4.73 (app t, 1H, $J \approx 5$ Hz), 2.89 (dd, 1H, J = 16, 4 Hz), 2.77 (dd, 1H, J = 17, 6.4 Hz), 1.60 (s, 3H), 1.56 (s, 3H).

S-*1,3-Dioxolane-4-acetamide, 2,2-dimethyl-5-oxo*-N-*[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl)]-* $C_{21}H_{30}N_2O_{12}$. A solution of *N*-3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucosylamine (0.485 g, 1.40 mmol) and the above acid (0.300 g, 1.74 mmol) were coupled using the standard $(EtO)_2P(O)CN$ procedure, and the residue was purified by silica chromatography to yield 0.393 g (0.83 mmol, 59 %) of the product: TLC R_f 0.31 (EtOAc); ^{1}H NMR ($CDCl_3$) : δ 7.15 (d, 1H, J = 8.2 Hz), 6.09 (d, 1H, J = 8.2 Hz), 5.10-4.95 (m, 2H), 4.65 (dd, 1H, J = 7, 3.5 Hz), 4.21 (dd, 1H, J = 12.5, 4.4 Hz), 4.12-3.98 (m, 2H), 3.70 (m, 1H, ω = 19 Hz), 2.78 (dd, 1H, J = 16.2, 3.2 Hz), 2.54 (dd, 1H, J = 16.2, 7.0 Hz), 2.02, 2.00, 1.98, 1.89, 1.55, 1.49 (all s, 3H); MS (FAB) m/z 503 ([M + H]$^+$; calcd 503), 461, 443, 414, 383, 365, 330, 210, 168, 150, 126, 108, 43.

Butanoic acid, 2S-hydroxy-4-oxo-4-[[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]- $C_{18}H_{26}N_2O_{12}$. The above material (0.39 g, 0.82 mmol) in glacial acetic acid (10 mL) and water (5 mL) was stirred at 45 °C for 2.5 h. The reaction was cooled, diluted with 100 mL water, and extracted thrice with 100 mL portions of EtOAc. The combined organics were back-extracted with water (4 × 100 mL). The combined aq. solutions were concentrated, and the residue azeotropically dried with several portions of toluene to provide, after vacuum drying, 0.33 g (0.71 mmol, 87 %) of the acid: MS (FAB) m/z 463.1556 ([M + H]$^+$; calcd 463.1564), 485, 421, 325, 224, 210, 168, 150,138, 126, 108, 43.

L-Histidinamide, N-*[4-[[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-2-hydroxy-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl*-N-*[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]*-N$^\alpha$-*methyl-1-[(4-methylphenyl)sulfonyl],* N-*oxide [1S[1R*(R*),2R*,4R*(1R*,2R*)]]* $C_{70}H_{97}N_{11}O_{20}S$. The above acid (0.243 g, 0.52 mmol) and the protected peptide **29** (0.42 g, 0.42 mmol) were coupled using the standard $(EtO)_2P(O)CN$ procedure,

to provide after silica flash chromatography 0.31 g (0.21 mmol, 50 %) of the protected glycopeptide: TLC R_f 0.26 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1444.7 ($[M + H]^+$; calcd 1444.7), 1466.7, 278.1, 154.1.

L-Histidinamide, N-[4-[[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-2-hydroxy-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nα-methyl, N-oxide [1S[1R(R*),2R*,4R*(1R*,2R*)]]* (**18**) $C_{57}H_{85}N_{11}O_{15}$. The protected glycopeptide (0.30 g, 0.21 mmol) in MeOH (6 mL) was treated with 7 M NH_3 in MeOH (12 mL) for 18 h. The reaction was concentrated, and the residue was purified by silica flash chromatography to yield 0.18 g (0.15 mmol, 74%) of the glycopeptide **18**: TLC R_f 0.11 (6:4 CH_2Cl_2:5 M NH_3 in MeOH); MS (FAB) m/z 1164.632 ($[M + H]^+$; calcd 1164.630), 1148, 582, 365, 204, 168, 124, 109, 86, 70.

*Preparation of glycopeptide **19***

Butanoic acid, (2,3-bis-(benzoyloxy)-4-oxo-[4-[[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]]-[S-(R,S*)] $C_{32}H_{34}N_2O_{15}$.* A solution of *meso*-dibenzoyltartaric acid (0.753 g, 2.0 mmol) in benzene (8 mL) was cyclized to the anhydride with refluxing CH_3COCl (1.5 mL) over 20 h.[34] The solvent was removed to give a white residue, which was reacted with 3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosylamine (0.724 g, 2.0 mmol) in CH_2Cl_2 (15 mL) at ambient temperature under Ar. The reaction was stirred for 20 h, and then was concentrated to provide, as a foam, 1.39 g (2.0 mmol, 100 %) of the glycosylamide acid: ^1H NMR ($CD_3S(O)CD_3$) : δ 8.88 (d, 1H, J = 9 Hz), 7.99 (d, 4H, J = 7.5 Hz), 7.67 (t, 2H, J = 7.2 Hz), 7.53 (q, 4H, J = 7.6 Hz), 5.82 (d, 1H, J = 1 Hz), 5.61 (d, 1H, J = 1 Hz), 5.27 (1:2:1 t, 1H, J = 9.0 Hz; after D_2O d, J = 10.0 Hz), 5.22 (1:2:1 t, 1H, J = 9.0 Hz), 4.87 (1:2:1 t, 1H, J = 9 Hz), 4.40-3.85 (m, 3H), 2.00, 1.98, 1.91, 1.70 (s, 3H); MS (FAB) m/z 687 ($[M + H]^+$), 616, 549, 330, 210, 168, 150, 126, 105, 43.

L-Histidinamide, N-[4-[[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxo-(2,3-di-(benzoyl)oxy)-butyl]-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nα-methyl, N-oxide [1S[1R(S*,R*),2R*,4R*(1R*,2R*)]] $C_{84}H_{105}N_{11}O_{23}S$.* The above glycosylamide acid (0.558 g, 0.81 mmol) and the protected peptide **29** (0.65 g, 0.65 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions. The product was purified by silica flash chromatography to provide 0.383 g (0.23 mmol, 35 %) of the protected glycopeptide: TLC R_f 0.54 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1668.8 ($[M + H]^+$; calcd 1668.7), 1104.6, 519.3, 358.4, 278.2, 174.1, 125.1.

L-Histidinamide, N-[4-[[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxo-2,3-(dihydroxybutyl]-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nα-methyl, N-oxide [1S[1R(S*,R*),2R*,4R*(1R*,2R*)]] (**19**) $C_{57}H_{85}N_{11}O_{16}$.* The above protected glycopeptide (0.363 g, 0.23 mmol) in MeOH (6 mL) was treated with 5 M NH_3 in MeOH (25 mL) for 18 h. The reaction was concentrated, and the product purified by silica flash chromatography, to provide 0.078 g (0.066 mmol, 29 %) of glycopeptide **19**: TLC R_f 0.17 (60:36:4 CH_2Cl_2:MeOH:satd aq. NH_3); MS (FAB) m/z 1180.626 ($[M + H]^+$; calcd 1180.625), 1164, 1005, 984, 950, 916, 231, 124, 109.

*Preparation of glycopeptide **20***

Butanoic acid, [2,3-bis-(benzoyloxy)-4-oxo-[4-[[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]]-[S-(R,R*)] $C_{32}H_{34}N_2O_{15}$.* A solution of dibenzoyl-D-tartaric acid (0.753 g, 2.0 mmol) in benzene (8 mL) was cyclized to the anhydride with refluxing CH_3COCl (1.5 mL) for 20 h. The solvent was removed to give a white residue, which was reacted with 3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosylamine (0.724 g, 2.0 mmol) in 15 mL CH_2Cl_2 at ambient temperature under Ar, and the reaction stirred for 20 h. The solution was concentrated to provide, as a foam, the product: ^1H NMR ($CD_3S(O)CD_3$) : δ 8.00 (d, 4H, J = 7.1 Hz), 7.83 (d, 1H, J = 9.1 Hz), 7.71 (m, 2H), 7.58 (q, 4H, J = 7 Hz), 5.89 (d, 1H, J = 2 Hz), 5.81 (d, 1H, J = 2 Hz), 5.17 (1:2:1 t, 1H, J = 9.5 Hz; after D_2O: d, J = 9.8 Hz), 5.05 (1:2:1 t, 1H, J = 9.9 Hz), 4.83 (1:2:1 t, 1H, J = 9.7 Hz), 4.18 (dd, 1H, J = 13, 2 Hz), 3.96-3.78 (m, 2H), 1.96, 1.94, 1.88, 1.46 (s, 3H); MS (FAB) m/z 687.2055 ($[M + H]^+$; calcd 687.2037), 676, 645, 627, 616, 549, 330, 210, 168, 150, 126, 105, 43.

L-Histidinamide, N-[4-[[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxo-[2,3-di-(benzoyl)oxy)-butyl]-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nα-methyl, N-oxide [1S[1R(R*,R*),2R*,4R*(1R*,2R*)]] $C_{84}H_{105}N_{11}O_{23}S$.* The above acid (0.50 g, 0.50 mmol) and the protected peptide **29** (0.429 g, 0.63 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions. The product was purified by silica flash chromatography to yield 0.309 g (0.185 mmol, 37 %)) of the protected glycopeptide: TLC R_f 0.57 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1668.7 ($[M + H]^+$; calcd 1668.8), 1514.7, 1138.5, 517.3, 330.1, 278.1, 210.1, 125.1.

L-Histidinamide, N-[4-[[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxo-2,3-(dihydroxybutyl]-L-prolyl-L-phenylalanyl-N-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-Nα-methyl, N-oxide [1S[1R(S*,R*),2R*,4R*(1R*,2R*)]] (**20**) $C_{57}H_{85}N_{11}O_{16}$.* The protected glycopeptide (0.30 g, 0.18 mmol) in MeOH (3 mL) was treated with 5 M NH_3 in MeOH (12 mL) for 44 h. The reaction was concentrated, and the residue was purified by silica flash chromatography to provide 0.100 g (0.085

mmol, 47 %) of glycopeptide **20**: TLC R_f 0.22 (60:36:4 CH_2Cl_2:MeOH:satd aq. NH_3); MS (FAB) m/z 1180 ($[M + H]^+$; calcd 1180), 1164, 365, 229, 204, 184, 156, 124, 108, 86, 70.

Preparation of glycopeptide ***21***

*Butanoic acid, 4-oxo-4-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1-(phenylmethyl) ester* $C_{25}H_{32}N_2O_{11}$. A solution of the glycosylamine (0.506 g, 1.42 mmol) and succinic acid monobenzyl ester (0.381 g, 1.83 mmol) were coupled using the standard $(EtO)_2P(O)CN$ conditions. The residue was purified by silica chromatography to yield 0.291 g (0.54 mmol, 38 %) of the glycosylamide ester: TLC R_f 0.25 (4:1 CH_2Cl_2:acetone; MS (FAB) m/z 537.2076 ($[M + H]^+$; calcd 537.2084), 477, 399, 371, 330, 210, 168, 150, 126, 108, 91, 43.

*Butanoic acid, 4-oxo-4-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-* $C_{18}H_{26}N_2O_{11}$. A suspension of the above ester (0.287 g, 0.53 mmol) and 0.082 g 10 % Pd:C catalyst in MeOH (40 mL) was hydrogenated at 40 psi for 1.5 h. The solution was filtered and concentrated to provide 0.228 g (0.51 mmol, 96 %) of the glycosylamide acid. MS (FAB) m/z 447 ($[M + H]^+$; calcd 447), 330, 283, 210, 168, 150, 43.

*L-Histidinamide, N-[4-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N^{α}*-methyl [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{63}H_{91}N_{11}O_{16}$. A solution of the tosyl-deprotected derivative of peptide **28** (0.284 g, 0.34 mmol) and the above acid (0.191 g, 0.43 mmol) were coupled using the standard $(EtO)_2P(O)CN$ conditions. The reaction was concentrated, and the residue was purified by silica chromatography to yield 0.290 g (0.23 mmol, 68 %) of the protected glycopeptide. TLC R_f 0.38 (9:1 CH_2Cl_2:4.5 M NH_3 in MeOH); MS (FAB) m/z 1258.669 ($[M + H]^+$; calcd 1258.672), 478, 358, 327, 299, 210, 197, 168, 150, 124, 109, 86, 70, 43.

*L-Histidinamide, 1-[4-[[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N^{α}*-methyl-[1S[1R*,2R*,4R*(1R*,2R*)]]* ***(21)*** $C_{57}H_{85}N_{11}O_{13}$. The protected glycopeptide (0.283 g, 0.225 mmol) in MeOH (6 mL) was treated with 4.5 M NH_3 in MeOH (3 mL) for 18 h. The reaction was concentrated, and the residue was purified by silica flash chromatography to yield 0.216 g (0.19 mmol, 85 %) of glycopeptide **21**: TLC R_f 0.26 (7:3 CH_2Cl_2:4.5 M NH_3 in MeOH); MS (FAB) m/z 1132.639 ($[M + H]^+$; calcd 1132.641), 586, 478, 365, 327, 299, 222, 204, 152, 124, 109, 86, 70.

Preparation of glycopeptide ***22***

*L-Histidinamide, N-[4-[[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]-hexyl]-*N^{α}*-methyl-1-[(4-methylphenyl)sulfonyl]-,* N*-oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{70}H_{97}N_{11}O_{19}S$. A solution of the protected peptide **29** (0.409 g, 0.409 mmol) and 4-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-4-oxobutanoic acid (0.228 g, 0.51 mmol) were coupled using the standard $(EtO)_2P(O)CN$ conditions. The reaction was concentrated, and the residue was purified by silica chromatography to yield 0.247 g (0.17 mmol, 42 %) of the protected glycopeptide: TLC R_f 0.58 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1428.6 ($[M + H]^+$; calcd 1428.7), 1274.6, 978.3, 429.1, 278.1, 210.1, 154.1.

*L-Histidinamide, 1-[4-[[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N^{α}*-methyl-,* N*-oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* ***(22)*** $C_{57}H_{85}N_{11}O_{14}$. The protected glycopeptide (0.242 g, 0.17 mmol) in MeOH (3 mL) was treated with 4 M NH_3 in MeOH (10 mL) for 18 h. The reaction was concentrated, and the residue was purified by silica flash chromatography to provide 0.174 g (0.15 mmol, 89 %) of glycopeptide **22**: TLC R_f 0.28 (7:3 CH_2Cl_2:5 M NH_3 in MeOH); MS (FAB) m/z 1170.612 ($[M + Na]^+$; calcd 1170.617), 1148 $[M + H]^+$, 1132, 1028, 973, 299, 231, 124, 70.

Preparation of glycopeptide ***23***

*Hexanoic acid, 6-oxo-6-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino], 1-(phenylmethyl) ester* $C_{27}H_{36}N_2O_{11}$. The glycosylamine (0.358 g, 1.04 mmol) and adipic acid monobenzyl ester (0.307 g, 1.30 mmol) were coupled using standard $(EtO)_2P(O)CN$ conditions. The reaction was concentrated, and the residue was purified by silica flash chromatography to yield 0.184 g (0.33 mmol, 31 %) of the glycosylamide ester: TLC R_f 0.35 (4:1 CH_2Cl_2:acetone); ^1H NMR ($CDCl_3$) : δ *inter alia* 5.35 (1:2:1 t, 1H, J = 10 Hz); MS (FAB) m/z 565.2376 ($[M + H]^+$; calcd 565.2397), 531, 475, 457, 427, 330, 236, 210, 168, 150, 91.

*Hexanoic acid, 6-oxo-6-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-* $C_{20}H_{30}N_2O_{11}$. A suspension of the above glycosylamide ester (0.18 g, 0.32 mmol) and 0.051 g 10 % Pd:C in MeOH (40 mL) was hydrogenated at 50 psi for 4 h. The solution was filtered and concentrated to provide 0.134 g (0.28 mmol, 88 %) of the glycosylamide acid: MS (FAB) m/z 475.1925 ($[M + H]^+$; calcd 475.1928), 433, 337, 295, 263, 229, 210, 199, 168.

*L-Histidinamide, 1,6-dioxo-*N*-[6-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]hexyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N^{α}*-methyl-1-[(4-methylphenyl)sulfonyl],* N*-oxide [1S*

[1R,2R*,4R*(1R*,2R*)]] $C_{72}H_{101}N_{11}O_{19}S$.* A solution of the glycopeptide **29** (0.223 g, 0.223 mmol) and the glycosylamide acid (0.132 g, 0.278 mmol) in CH_2Cl_2 (6 mL) were coupled under standard $(EtO)_2P(O)CN$ conditions. The reaction was concentrated, and the residue was purified by silica flash chromatography to give 0.127 g (0.087 mmol, 39 %) of the protected glycopeptide: TLC R_f 0.14 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1456.6 ($[M + H]^+$; calcd 1456.7).

*L-Histidinamide, 1-[6-[[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,6-dioxohexyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N$^{\alpha}$*-methyl, N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]] (23)* $C_{59}H_{89}N_{11}O_{14}$. A solution of the protected glycopeptide (0.125 g, 0.086 mmol) in MeOH (2 mL) was treated with 4 M NH_3 in MeOH (4 mL) for 18 h. The reaction was concentrated, and the residue was purified by silica flash chromatography to yield 0.091 g (0.077 mmol, 89%) of glycopeptide **23**: TLC R_f 0.19 (3:1 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1176 ($[M + H]^+$; calcd 1176).

Preparation of glycopeptide ***24***

Butanoic acid, 4-oxo-[4-[[3,6-di-O-acetyl-2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-β-D-glucopyranosyl]amino]]-1-(phenylmethyl) ester $C_{37}H_{49}N_3O_{18}$. Heptaacetylchitobiosylamine (0.80 g, 1.26 mmol) and succinic acid monobenzyl ester (0.329 g, 1.58 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions. The reaction was concentrated, and the residue was purified by silica flash chromatography to give 0.74 g (0.88 mmol, 70 %) of the diglycosylamide ester: TLC R_f 0.19 (1:1 acetone:CH_2Cl_2); MS (FAB) m/z 824 ($[M + H]^+$; calcd 824), 782, 734, 635, 617, 330, 228, 210, 168, 150, 138, 126, 108, 91, 43.

Butanoic acid, 4-oxo-[4-[[3,6-di-O-acetyl-2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-β-D-glucopyranosyl]amino]] $C_{30}H_{43}N_3O_{18}$. A solution of the above glycosylamide ester (0.734 g, 0.87 mmol), NH_4HCO_2 (0.827 g), and 5 % Pd/C catalyst (0.41 g) in DMF (13.6 mL) was stirred at ambient temperature under Ar for 20 h. The reaction was filtered and concentrated to give 0.64 g (0.87 mmol, 98 %) of the glycosylamide acid: TLC R_f 0.16 (4:1 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 734.2668 ($[M + H]^+$; calcd 734.2620), 657, 330, 228, 210, 168, 150, 138, 126, 108, 91, 43.

L-Histidinamide, N*-[4-[[3,6-di-O-acetyl-2-(acetylamino)-2-deoxy-4-O-[3,4,6-tri-O-acetyl-2-(acetyl-amino)-2-deoxy-β-D-glucopyranosyl]-β-D-glucopyrano-syl]amino]]-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]-carbonyl]hexyl]-*N$^{\alpha}$*-methyl-1-[(4-methylphenyl) sulfonyl], N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{82}H_{114}N_{12}O_{26}S$. The protected peptide **29** (0.67 g, 0.67 mmol) and the glycosylamide acid (0.63 g, 0.84 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions. The reaction was concentrated, and the residue was purified by silica flash chromatography to yield 0.36 g (0.21 mmol, 31 %) of the protected glycopeptide: TLC R_f 0.49 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1715.8 ($[M + H]^+$; calcd 1715.8), 1737.7 $[M + Na]^+$; 1753.6 $[M + K]^+$.

L-Histidinamide, N*-[4-[[2-(acetylamino)-2-deoxy-4-O-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-β-D-glucopyranosyl]amino]]-1,4-dioxobutyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]-carbonyl]butyl]amino]carbonyl]hexyl]-*N$^{\alpha}$*-methyl, N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]] (24)* $C_{65}H_{98}N_{12}O_{19}$. The protected glycopeptide (0.356 g, 0.21 mmol) in MeOH (6 mL) was treated at ambient temperature under Ar with 4 M NH_3 in MeOH (10 mL). After 20 h the yellow solution was concentrated, and the residue was purified by silica chromatography to give 0.183 g (0.135 mmol, 65 %) of glycopeptide **24**: TLC R_f 0.18 (6:4 CH_2Cl_2:5 M NH_3 in MeOH); MS (FAB) m/z 1351 ($[M + H]^+$; calcd 1351), 1335, 1176, 1130, 478, 327, 299, 124, 70.

Preparation of glycopeptide ***25***

L-Histidinamide, N*-[3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-*N^2*-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N$^{\alpha}$*-methyl-1-[(4-methylphenyl)sulfonyl]-[1S[1R*,2R*,4R*(1R*,2R*)]] [125907-05-3]* $C_{70}H_{99}N_{11}O_{19}S$. The glycosylasparagine (0.17 g, 0.30 mmol) and protected peptide **31** (0.21 g, 0.24 mmol) were coupled under standard $(EtO)_2P(O)CN$ conditions. The reaction was concentrated, and the residue was purified by silica flash chromatography (96:4 CH_2Cl_2:MeOH) to provide 0.22 g (0.16 mmol, 65 %) of the protected glycopeptide: TLC R_f 0.49 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1431 ($[M + H]^+$; calcd 1431), 1277, 997, 741, 587, 519, 488, 453, 365, 330.

L-Histidinamide, N*-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-*N^2*-[(1,1-dimethylethoxy)carbonyl]-L-asparaginyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinyl-methyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N$^{\alpha}$*-methyl-1-[(4-methylphenyl)sulfonyl]-[1S[1R*, 2R*,4R*(1R*,2R*)]] (25)* $C_{57}H_{87}N_{11}O_{14}$. The above glycopeptide (0.22 g, 0.153 mmol) in MeOH (7.5 mL) was treated at ambient temperature under Ar with 0.5 M aq. KOH (0.5 mL). The reaction was concentrated after 2 h, and was partitioned between brine (60 mL) and CH_2Cl_2 (60 mL). The emulsion was clarified by centrifugation. The glycopeptide was found as a white solid at the interface. It was collected by filtration and then dried under vacuum to give 0.164 g (0.143 mmol, 93 %) of **25**: TLC R_f 0.16 (4:1 CH_2Cl_2:4 M NH_3 in MeOH); MS (FAB) m/z 1150 ($[M + H]^+$, calcd 1150), 586, 365, 222, 204, 177, 138, 124, 109.

Preparation of glycopeptide **26**

*L-Histidinamide, 1,6-dioxo-*N*-[6-[[3,4,6-tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-hexyl]-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N*$^{\alpha}$-methyl-1-[(4-methylphenyl)sulfonyl], N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{67}H_{94}N_{10}O_{18}S$. A solution of the protected peptide **32** (0.37 g, 0.41 mmol) and the glycosylamide acid (0.243 g, 0.51 mmol) were coupled using standard $(EtO)_2P(O)CN$ conditions. The residue was purified by silica chromatography to yield 0.206 g (0.15 mmol, 37 %) of the protected glycopeptide: TLC R_f 0.40 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1359 ($[M + H]^+$; calcd 1359), 1342, 1235, 1205, 1189, 519, 278, 230, 210, 168, 150, 124, 108.

*L-Histidinamide, 1-[6-[[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]amino]-1,6-dioxohexyl]-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N*$^{\alpha}$-methyl, N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]] (26)* $C_{54}H_{82}N_{10}O_{13}$. A solution of the protected glycopeptide (0.20 g, 0.147 mmol) in MeOH (4 mL) was treated with 5 M NH_3 in MeOH (10 mL) for 18 h. The reaction was concentrated, and the residue purified by silica flash chromatography to yield 0.137 g (0.127 mmol, 86 %) of glycopeptide **26**: TLC R_f 0.09 (4:1 CH_2Cl_2:5 M NH_3 in MeOH); MS (FAB) m/z 1079.616 ($[M + H]^+$; calcd 1079.614), 1063, 602, 365, 230, 204, 172, 124, 109, 86, 69, 55.

Preparation of glycopeptide **27**

*β-D-Glucopyranose, 2-(acetylamino)-1,2-dideoxy-1-thio-3,4,6-tri-*O*-acetyl-1-carbaimidate, monohydrochloride [51224-18-1]* $C_{15}H_{23}N_3O_8S{\cdot}HCl$. A solution of 2-(acetylamino)-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylchloride (16.6 g, approx. 33 mmol) and thiourea (3.71 g, 48.7 mmol) in acetone was refluxed for 30 min, and then cooled. After standing for 18 h the white precipitate was collected, washed with cold acetone and ether, and vacuum-dried to provide 10.2 g (23 mmol, 70 %) of the product as a bright white, non-hygroscopic solid.

*Hexanoic acid, [6-[[2-(acetylamino)-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]thio]-* $C_{20}H_{31}NO_{10}S$. The above pseudothiourea salt (1.55 g, 3.5 mmol), $NaHSO_3$ (0.35 g), and K_2CO_3 (0.625 g, 4.5 mmol) were partitioned between water (7.5 mL) and $CHCl_3$ (7.5 mL), and the mixture stirred vigorously at ambient temperature for 45 min. The organic layer was separated, and the aqueous solution extracted with two additional $CHCl_3$ portions. The combined organics were concentrated to give the crude glycosylthiol (1.34 g) as a white foam. To this was added immediately 6-bromohexanoic acid (0.755 g, 3.87 mmol), K_2CO_3 (1.04 g, 7.5 mmol), and MeOH (10 mL) under N_2. The solids dissolved within several minutes. Shortly thereafter a white solid reappeared. After 20 h, formic acid (1 mL) was added cautiously. The mixture was partitioned between EtOAc (50 mL) and 0.5 M aq. HCl (20 mL). The aqueous layer was washed with two additional EtOAc portions. The combined organics were dried and concentrated. The residue was purified by silica chromatography to give 0.85 g (1.77 mmol, 51 %) of the product: TLC R_f 0.54 (7:3:0.1 CH_2Cl_2:acetone: HCO_2H); ^1H NMR ($CDCl_3$) : δ *inter alia* 5.82 (br d, 1H, J = 9.4 Hz), 5.18 (app t, 1H, J = 9.6 Hz), 5.10 (app t, 1H, J = 9.5 Hz), 4.59 (d, 1H, J = 10.3 Hz), 4.24 (dd, 1H, J = 11.5, 4.8 Hz), 4.12 (m, 2H), 4.12 (m, 2H), 3.70 (m, 1H), 2.71 (m, 2H), 2.36 (t, 2H, J = 7.3 Hz), 2.09 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.64 (m, 4H), 1.45 (m, 2H); ^{13}C NMR ($CDCl_3$) : δ *inter alia* CH: 84.34, 75.83, 73.91, 68.46, 53.22; CH_2: 62.38, 33.81, 29.61, 29.04, 28.00, 24.19; CH_3: 23.18, 20.78, 20.73, 20.64.

*L-Histidinamide, 1-[6-[[2-(acetylamino)-2-deoxy- 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]thio]hexyl]-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N*$^{\alpha}$-methyl-1-[(4-methylphenyl)sulfonyl]-, N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{67}H_{95}N_9O_{17}S_2$. The protected peptide **32** (0.239 g, 0.26 mmol) and the above glycosylthioacid (0.16 g, 0.33 mmol) were coupled using standard $(EtO)_2P(O)CN$ conditions. The residue was purified by silica chromatography to give 0.14 g (0.10 mmol, 39 %) of the protected glycopeptide: TLC R_f 0.31 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1362.6 ($[M + H]^+$; calcd 1362.6), 1238.4, 1208.6, 912.3, 756.4, 519.3.

*L-Histidinamide, 1-[6-[[2-(acetylamino)-2-deoxy-3,4,6-β-D-glucopyranosyl]thio]hexyl]-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N*$^{\alpha}$-methyl-, N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]] (27)* $C_{54}H_{83}N_9O_{12}S$. A solution of the protected glycopeptide (0.135 g, 0.099 mmol) in MeOH (2 mL) was treated with 5 M NH_3 in MeOH (5 mL) for 18 h. The reaction was concentrated, and the residue purified by silica flash chromatography to yield 0.102 g (0.091 mmol, 92 %) of glycopeptide **27**: TLC R_f 0.16 (4:1 CH_2Cl_2:5 M NH_3 in MeOH); MS (FAB) m/z 1082 ($[M + H]^+$; calcd 1082), 1066, 955, 877, 845, 204, 144, 124, 95, 86.

Preparation of peptide **29**

*L-Histidinamide,1-[(1,1-dimethylethoxy)carbonyl]-L-prolyl-L-phenylalanyl-*N*-[2-hydroxy-5-methyl-1-(2-methylpropyl)-4-[[[2-methyl-1-[[(2-pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]-*N*$^{\alpha}$-methyl-1-[(4-methylphenyl)sulfonyl]-N-oxide [1S[1R*,2R*,4R*(1R*,2R*)]] [130985-35-2]* $C_{57}H_{81}N_9O_{11}S$. A solution of the protected peptide (2.72 g, 2.51 mmol) and 3-$ClPhCO_3H$ (1.83 g) in $CHCl_3$ (110 mL) was stirred at ambient temperature under Ar for 2 h. The reaction mixture was poured into ice-cold 10 % $NaSO_3$, and then extracted with $CHCl_3$ (4 × 250 mL). The combined organics were dried, filtered, and concentrated to give 2.70 g (2.45 mmol, 97 %) of the peptide

N–oxide. This was carried forward for N–deprotection without further purification. TLC R_f 0.28 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1100 ($[M + H]^+$; calcd 1100), 1084, 976, 946, 930, 650, 519, 278, 261, 217.

*L–Histidinamide, L–prolyl–L–phenylalanyl–*N*–[2–hydroxy–5–methyl–1–(2–methylpropyl)–4–[[[2–methyl–1–[[(2–pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]–*N*$^{\alpha}$*–methyl–1–[(4–methylphenyl)sulfonyl]–*N*–oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* (**29**) $C_{52}H_{73}N_9O_9S$. A solution of the above N–terminus Boc–protected peptide (3.20 g) in 2:1 CH_2Cl_2:CF_3CO_2H (30 mL) at ambient temperature under Ar was stirred for 2 h. The reaction mixture was poured into ice–cold satd aq. $NaHCO_3$ (250 mL), and extracted with $CHCl_3$ (4 × 250 mL). The organics were dried, filtered and concentrated to give 2.13 g (0.213 mmol, 85 %) of the deprotected peptide **29** as a foam.

Preparation of peptide 32

*L–Histidinamide,1–[(1,1–dimethylethoxy)carbonyl]–L–phenylalanyl–*N*–[2–hydroxy–5–methyl–1–(2–methylpropyl)–4–[[[2–methyl–1–[[(2–pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]–*N*$^{\alpha}$*–methyl–1–[(4–methylphenyl)sulfonyl]–*N*–oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* $C_{52}H_{74}N_8O_{10}S$. A solution of **30** (0.965 g, 0.98 mmol) and 3–$ClPhCO_3H$ (0.81 g) in $CHCl_3$ (40 mL) was stirred at ambient temperature under Ar for 1.5 h. The reaction mixture was poured into ice–cold 10 % $NaSO_3$ (150 mL), and then extracted with $CHCl_3$ (4 × 150 mL). The combined organics were dried, filtered, and concentrated to give 0.95 g (0.94 mmol, 96 %) of the peptide N–oxide. This was carried forward for N–deprotection without purification. TLC R_f 0.57 (9:1 CH_2Cl_2:MeOH); MS (FAB) m/z 1003.535 ($[M + H]^+$; calcd 1003.533), 987, 879, 865, 849, 833, 453, 299, 278, 124, 108, 86, 69, 57.

*L–Histidinamide, L–phenylalanyl–*N*–[2–hydroxy–5–methyl–1–(2–methylpropyl)–4–[[[2–methyl–1–[[(2–pyridinylmethyl)amino]carbonyl]butyl]amino]carbonyl]hexyl]–*N*$^{\alpha}$*–methyl–1–[(4–methylphenyl)sulfonyl]–*N*–oxide [1S[1R*,2R*,4R*(1R*,2R*)]]* (**32**) $C_{47}H_{66}N_8O_8S$. A solution of the above N–terminus Boc–protected peptide (1.08 g, 0.98 mmol) in 3:1 CH_2Cl_2:CF_3CO_2H (13.5 mL) at ambient temperature under Ar was stirred for 2 h. The reaction mixture was poured into ice–cold satd aq. $NaHCO_3$ (150 mL), and extracted with $CHCl_3$ (4 × 150 mL). The organics were dried, filtered and concentrated to give 0.626 g (0.69 mmol, 72 %) of the deprotected peptide **32**: TLC R_f 0.41 (9:1 CH_2Cl_2: MeOH); MS (FAB) m/z 903.480 ($[M + H]^+$; calcd 903.480), 887, 749, 453, 299, 278, 238, 196, 125, 109, 86, 69.

References and Notes

1. Plattner, J. J.; Norbeck, D. W. *Drug Discovery Technologies*; pp. 92–128, Clark, C. R; Moos, W. H., Eds.; Ellis Harwood–Halsted Press: Chichester, 1989.

2. (a) Bertrams, A. A.; Ziegler, K. *Biochim. Biophys. Acta* **1991**, *1073*, 213. (b) Elstner, R. H.; Ziegler, K. *J. Biol. Chem.* **1992**, *267*, 9788. (c) Ziegler, K.; Sanger, U. *Biochim. Biophys. Acta* **1992**, *1103*, 219. (d) Ziegler, K.; Seeberger, A. *Biochem. Pharmacol.* **1993**, *45*, 909. (e) Seeberger, A.; Ziegler, K. *Biochem. Pharmacol.* **1993**, *45*, 917. (f) Hamilton, H.; Steinbaugh, B.; Blankley, J.; Taylor, M.; Chan, O. H.; Stewart, B.; Schroeder, R.; Ryan, M.; Rapundalo, S.; Cook, J.; Bernabei, A.; Stewart, C. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 813.

3. (a) Thaisrivongs, S.; Pals, D. T., Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088. (b) Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; DeGraaf, G. L.; Harris, D. W.; Johnson, G. A. *Hypertension* **1986**, *8*, 1105. (c) Kati, W. M.; Pals, D. T.; Thaisrivongs, S. *Biochemistry* **1987**, *26*, 7621. (d) Epps, D. E.; Cheney, J.; Schostarez, H. J.; Sawyer, T. K.; Prairie, M.; Krueger, W. C.; Mandel, F. *J. Med. Chem.* **1990**, *33*, 2080. (e) Epps, D. E.; Poorman, R. A.; Mandel, F.; Schostarez, H. J. *J. Med. Chem.* **1991**, *34*, 2107.

4. Renin modulation of blood pressure occurs *inter alia* in the plasma, vasculature, heart, and kidney: Laragh, J. H. *Hypertension* **1992**, *20*, 267. Paul, M.; Bachmann, J.; Ganten, D. *Trends Cardiovasc. Med.* **1992**, *2*, 94. Johnston, C. I. *J. Hypertens.* **1992**, *10* (suppl 7), S13. Dostal, D. E.; Baker, K. M. *Trends Cardiovasc. Med.* **1993**, *3*, 67. Dzau, V. J. *Arch. Intern. Med.* **1993**, *153*, 937. Laragh, J. H. *Arzneim.–Forsch.* **1993**, *43*, 247. Cody, R. J. *Drugs* **1994**, *47*, 586.

5. (a) Rush, B. D.; Wilkinson, K. F.; Zhong, W. Z.; Closson, S. K.; Lakings, D. B.; Ruwart, M. J. *Int. J. Pharmaceut.* **1991**, *73*, 231. (b) Greenfield, J. C.; Cook, K. J.; O'Leary, I. A. *Drug Metab. Dispos.* **1989**, *17*, 518. (c) Adedoyin, A.; Perry, P. R.; Wilkinson, G. R. *Drug Metab. Dispos.* **1993**, *21*, 184.

6. Reviews: Ocain, T. D.; Abou–Gharbia, M. *Drugs Future* **1991**, *16*, 37. Baldwin, J. J. *Curr. Opin. Therapeut. Patents* **1993**, *137*. Siegl, P. K. S.; Greenlee, W. J. *Curr. Opin. Invest. Drugs* **1993**, *2*, 931.

7. Abbott: Kleinert, H. D.; Stein, H. H.; Boyd, S.; Fung, A. K. L.; Baker, W. R.; Verburg, K. M.; Polakowski, J. S.; Kovar, P.; Barlow, J.; Cohen, J.; Klinghofer, V.; Mantei, R.; Cepa, S.; Rosenberg, S.; Denissen, J. F. *Hypertension* **1992**, *20*, 768. Kleinert, H. D.; Rosenberg, S. H.; Baker, W. R.; Stein, H. H.; Klinghofer, V.; Barlow, J.; Spina, K.; Polakowski, J.; Kovar, P.; Cohen, J.; Denissen, J. F. *Science* **1992**, *257*, 1940. Wessale, J. W.; Calzadilla, S. V.; Boyd, S. A.; Baker, W. R.; Stein, H. H.; Kovar, P. J.; Barlow, J.; Klinghofer, V.; Mantei, R.; Kleinert, H. D. *J. Cardiovasc. Pharmacol.* **1993**, *22*, 644. Gupta, S. K.; Granneman, G. R.; Packer, M.; Boger, R. S. *J. Cardiovasc. Pharm.* **1993**, *21*, 834. Verburg, K. M.; Polakowski, J. S.; Kovar, P. J.; Klinghofer, V.; Barlow, J. L.; Stein, H. H.; Mantei, R. A.; Fung, A. K. L.; Boyd, S. A.; Baker, W. R.; Kleinert, H. D. *J. Cardiovasc. Pharm.* **1993**, *21*, 149. Rosenberg, S. H.; Spina, K. P.; Woods, K. W.; Polakowski, J.; Martin, D. L.; Yao, Z.; Stein, H. H.; Cohen, J.; Barlow, J. L.; Egan, D. A.; Tricarico, K. A.; Baker, W. R.; Kleinert, H. D. *J. Med. Chem.* **1993**, *36*, 449.

8. Ciba–Geigy: Wood, J. M.; Jobber, R. A.; Baum, H.–P.; de Gasparo, M.; Nussberger, J. *J. Hypertens.* **1989**, *7*, 615.

9. Hoffmann–La Roche: Doig, J. K.; MacFadyen, R. J.; Meredith, P. A.; Fischli, W.; Reid, J. L. *J. Cardiovasc. Pharm.* **1992**, *20*, 875. Clozel, J.–P.; Fischli, W. *Arzneim.–Forsch.* **1993**, *43*, 260. Clozel, J.–P.; Fischli, W. *Hypertension* **1993**, *21*, 9. van den Meiracker, A. H.; Admiraal, P. J. J.; Derkx, F. H. M.; Kleinbloesem, C.; Man in 't Veld, A. J.; van Brummelen, P.; Mulder, P.; Schalekamp, M. A. D. H. *J. Hypertens.* **1993**, *11*, 831. Kobrin, I.; Viskoper, R. J.;

Laszt, A.; Bock, J.; Weber, C.; Charlton, V. *Am. J. Hypertens.* **1993**, *6*, 349. Weber, C.; Birnböck, H.; Leube, J.; Kobrin, I.; Kleinbloesem, C. H.; van Brummelen, *P. Br. J. Clin. Pharmacol.* **1993**, *36*, 547.

10. Parke–Davis: Patt, W. C.; Hamilton, H. C.; Ryan, M. J.; Painchaud, C. A.; Taylor, M. D.; Rapundalo, S. T.; Batley, B. L.; Connolly, C. J. C.; Taylor, D. G., Jr. *Med. Chem. Res.* **1992**, *2*, 10. Pfizer: Fossa, A. A.; Weinberg, L. J.; Barber, R. L.; Rauch, A. L.; Nocerini, M. R., Murphy, W. R.; Swindell, A. C. *J. Cardiovasc. Pharmacol.* **1992**, *20*, 75.

11. Sanofi: Denolle, T.; Luo, P.; Guyene, T. T.; Cazaubon, C.; Sissmann, J.; Corvol, P.; Ménard, J. *Arzneim.–Forsch.* **1993**, *43*, 255.

12. Warner–Lambert: Ryan, M. J.; Hicks, G. W.; Batley, B. L.; Rapundalo, S. T.; Patt, W. C.; Tatlor, D. G.; Keiser, J. A. *J. Pharmacol. Exp. Therapeut.* **1994**, *268*, 372. Yamanouchi: Shibasaki, M.; Usui, T.; Inagaki, O.; Asano, M.; Takenaka, T. *J. Pharm. Pharmacol.* **1994**, *46*, 68.

13. Upjohn: (a) Bundy, G. L.; Pals, D. T.; Lawson, J. A.; Couch, S. J.; Lipton, M. F.; Mauragis, M. A. *J. Med. Chem.* **1990**, *33*, 2276. (b) Thaisrivongs, S.; Pals, D. T.; DuCharme, D. W.; Turner, S. R.; DeGraaf, G. L.; Lawson, J. A.; Couch, S. J.; Williams, M. V. *J. Med. Chem.* **1991**, *34*, 633.

14. Pals, D. T.; Lawson, J. A.; Couch, S. J. *J. Pharmacol. Meth.* **1990**, *23*, 239.

15. Kobata, A. *Eur. J. Biochem.* **1992**, *209*, 483. Stockwell Hartree, A.; Renwick, A. G. C. *Biochem. J.* **1992**, *287*, 665. Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1.

16. Kunz, H. *Pure Appl. Chem.* **1993**, *65*, 1223.

17. Fisher, J. F.; Harrison, A. W.; Bundy, G. L.; Wilkinson, K. F.; Rush, B. D.; Ruwart, M. J. *J. Med. Chem.* **1991**, *34*, 3140.

18. Paulsen, H.; Györgydeák, Z.; Friedmann, M. *Chem. Ber.* **1974**, *107*, 1590. Györgydeák, Z.; Szilágyi, L. *Liebigs Ann. Chem.* **1986**, 1393. Györgydeák, Z.; Szilágyi, L.; Paulsen, H. *J. Carbohydr. Chem.* **1993**, *12*, 139. β–Azides are Configurationally Stable upon Reduction to the Glycosylamine: Ogawa, T.; Nakabayashi, S.; Shibata, S. *Agric. Biol. Chem.* **1983**, *47*, 281.

19. Nakabayashi, S.; Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1988**, *174*, 279. Kunz, H.; Waldmann, H.; März, J. *Liebigs Ann. Chem.* **1989**, 45.

20. Lee, H. H.; Baptista, J. A. B.; Krepinsky, J. J. *Can. J. Chem.* **1990**, *68*, 953. Petö, C.; Batta, G.; Györgydeák, Z.; Sztaricskai, F. *Liebigs Ann. Chem.* **1991**, 505. Kunz, H.; Pfrengle, W.; Rück, K.; Sager, W. *Synthesis* **1991**, 1039. Unverzagt, C.; Kunz, H. *J. Prakt. Chem.* **1992**, *334*, 570.

21. (a) Christiansen–Brams, I.; Meldai, M.; Bock, K. *J. Chem. Soc. Perkin 1*, **1993**, 1461. (b) Lee, J.; Coward, J. K. *J. Org. Chem.* **1992**, *57*, 4126.

22. Lee and Coward[21b] note that NH_3/MeOH is sometimes unreliable for deacetylation of disaccharide glycosylasparagines. We did not encounter difficulty.

23. Urge, L.; Otvos, L., Jr.; Lang, E.; Wroblewski, K.; Laczko, I.; Hollosi, M. *Carbohydr. Res.* **1992**, *235*, 83.

24. Cho, M. J.; Scieszka, J. F.; Burton, P. S. *Int. J. Pharm.* **1989**, *52*, 79.

25. Kleeman, H.–W.; Heitsch, H.; Henning, R.; Kramer, W.; Kocher, W.; Lerch, U.; Linz, W.; Nickel, W.–U.; Ruppert, D.; Urbach, H.; Utz, R.; Wagner, A.; Weck, R.; Wiegand, F. *J. Med. Chem.* **1992**, *35*, 559. Boyd, S. A.; Fung, A. K. L.; Baker, W. R.; Mantei, R. A.; Armiger, Y.–L., Stein, H. H.; Cohen, J.; Egan, D. A.; Barlow, J. L.; Klinghofer, V.; Verburg, K. M.; Martin, D. L.; Young, G. A.; Polakowski, J. S.; Hoffman, D. J.; Garren, K. W.; Prun, T. J.; Kleinert, H. D. *J. Med. Chem.* **1992**, *32*, 1735.

26. Matsumoto, Y.; Ohsako, M.; Takadate, A.; Goto, S. *J. Pharm. Sci.* **1993**, *82*, 399.

27. Palomino, E.; Walker, E. H.; Blumenthal, S. L. *Drugs Future* **1991**, *16*, 1029.

28. Albert, R.; Marbach, P.; Bauer, W.; Briner, U.; Fricker, G.; Bruns, C. C; Pless, J. *Life Sci.* **1993**, *53*, 517.

29. The improved oral availability with *maltose* functionalization may relate to the phenomenon of improved solute availability coupled to brush border enzymatic (here, maltase) catalysis: Amidon, G. L.; Leesman, G. D.; Elliott, R. L. *J. Pharm. Sci.* **1980**, *69*, 1363. For two recent discussions: Pellicciari, R.; Garzon–Aburbeh, A.; Natalini, B.; Marinozzi, M.; Clerici, C.; Gentili, G.; Morelli, A. *J. Med. Chem.* **1993**, *36*, 4201. Lopez–Candales, A.; Bosner, M. S.; Spilburg, C. A.; Lange, L. G. *Biochemistry* **1993**, *32*, 12085.

30. The reader is directed to an astute appraisal of this term, and elegant demonstration of this concept: Polt, R.; Szabó, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114*, 10249.

31. Urge, L.; Gorbics, L.; Otvos, L., Jr. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 1125. Gerz, M.; Matter, H.; Kessler, H. *Ang. Chem. Int. Ed. Eng.* **1993**, *32*, 269. Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 3352.

32. Spimola, M.; Jeanloz, R. W. *J. Biol. Chem.* **1970**, *245*, 4158. Inaba, T.; Ohgushi, T.; Iga, Y.; Hasegawa, E. *Chem. Pharm. Bull.* **1984**, *32*, 1597. Nishimura, S.–I.; Kuzuhara, H.; Takiguchi, Y.; Shimahara, K. *Carbohydr. Res.* **1989**, *194*, 223.

33. Sterling, J.; Slovin, E.; Barasch, D. *Tetrahedron Lett.* **1987**, *28*, 1685.

34. Bell, K. H. *Aust. J. Chem.* **1987**, *40*, 399.

(*Received in U.S.A.* 4 *June* 1994; *accepted* 13 *July* 1994)