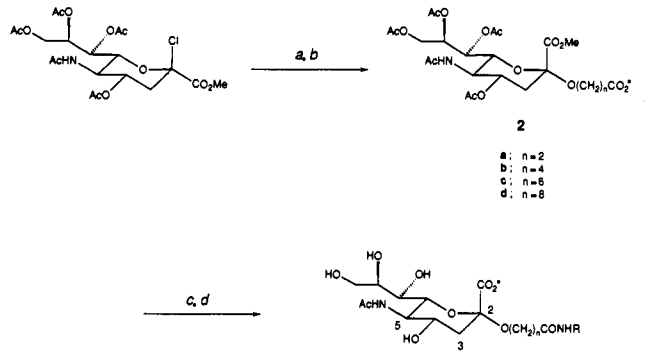
Scheme I. General Route to Monovalent Sialosides[a]



[a] (a) $HO(CH_2)_nCH{=}CH_2$ (as solvent), $Hg(CN)_2$ (0.1 equiv), 3-Å molecular sieves, room temperature; (b) $KMnO_4$ (3 equiv), AcOH(aq), 4 °C, 2 h; (c) 1-hydroxybenzotriazole (1 equiv), dicyclohexylcarbodiimide (1 equiv), diisopropylethylamine (1 equiv), RNH_2 (1 equiv), CH_2Cl_2; (d) methanol, NaOH(aq), room temperature, 2 h.

In several cases, binding to BHA was also measured by the NMR titration assay.[5] This method affords relative binding affinities that are in agreement with those obtained from hemagglutination inhibition experiments.

From Table I it can be seen that every compound that binds to HA better than Neu5Acα2Me (1) carries an aromatic group attached to the nitrogen of the aglycon amide linkage. This finding suggests that the aromatic group contributes to binding and that its positioning is critical for tight association with HA. The best inhibitors (13 and 14), with K_i = 40 μM, bind to HA more than 60-fold better than Neu5Acα2Me (K_i = 2.8 mM).[5] These are the tightest binding monovalent inhibitors of hemagglutination that have been described. It is interesting to note that the binding affinity is related to both the nature of the aglycon substituent (R) and the chain length (n). When R is the 1-naphthylmethyl group, the tightest binding inhibitor has the chain length n = 6. Examination of inhibitors modeled into the SA binding site of BHA does not reveal an obvious hydrophobic binding locus on the protein surface, and a proper understanding of the reason for the observed increases in binding affinity will have to await the solution of the crystal structure of a protein–ligand complex.

The identification of tight-binding sialosides containing aromatic substituents also suggests the possibility of synthesizing fluorescent ligands for BHA. This idea is currently being developed to provide a convenient fluorescence assay for the binding of ligands to influenza A hemagglutinin.

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Registry No. **2a**, 136067-24-8; **2b**, 136097-52-4; **2c**, 136067-25-9; **2d**, 119054-28-3; **3**, 136067-12-4; **4**, 136088-26-1; **5**, 136067-13-5; **6**, 136097-51-3; **7**, 136067-14-6; **8**, 136067-15-7; **9**, 136067-16-8; **10**, 136067-17-9; **11**, 136067-18-0; **12**, 136067-19-1; **13**, 136067-20-4; **14**, 136067-21-5; **15**, 136067-22-6; **16**, 136067-23-7; $MeNH_2$, 74-89-5; $PhCH_2NH_2$, 100-46-9; $3\text{-}(H_2NCH_2)C_6H_4COOH$, 2393-20-6; $PhCH_2OCONHCH_2CH_2NH_2$, 72080-83-2; (1-naphthylmethyl)amine, 118-31-0; (9-phenanthrylmethyl)amine, 15398-91-1; (5-dimethylamino-1-naphthyl)sulfonylhydrazine, 33008-06-9; (6-quinolinylmethyl)amine, 99071-54-2; methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-2-chloro-2-deoxy-β-D-neuraminate, 67670-69-3.

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Peptide to Glycopeptide: Glycosylated Oligopeptide Renin Inhibitors with Attenuated in Vivo Clearance Properties

Given the remarkable breadth of biological activity vested by Nature in the assembled amino acids, the value of peptides as preeminent templates for therapeutic drug design is self-evident. It is in recognition of this fact that considerable resources are expended, on a continuing basis, on the task of identifying chemical strategies that preserve the information content of the peptide and abolish its intrinsic shortcomings as a practical drug.[1] Of these shortcomings three are particularly vexing. The typical oligopeptide is susceptible to proteolytic degradation and is poorly orally absorbed, and of that modest fraction which is absorbed, there exists an efficient liver transport system[2] that extracts the oligopeptide from the blood and excretes it through the bile. It is within the context of this latter problem—liver/biliary clearance—that we report a distinct contrast in disposition between an oligopeptide and its glycosylated derivative.

A practical illustration of the difficulty posed by the therapeutic oligopeptide is provided by the inhibitors[3] of the aspartyl protease renin. This enzyme is an attractive target for the amelioration of hypertension, as a result of its specific proteolytic release of the oligopeptide precursor of the vasoconstrictive hormone angiotensinogen II. Di-

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tekiren[4] (1, U-71038) is a potent (K_i = 0.19 nM)[5] and proteolytically stable inhibitor of renin, but is without appreciable oral activity. Since the replacement of the amino and carboxy terminii of 1 by hydrophilic segments (exemplified by 2) results in a dramatic improvement in both aqueous solubility and oral activity,[6,7] it was thought that fashioning ditekiren as a glycopeptide might likewise result in salutary pharmacological properties. Glycosylation remains, after all, the rule rather than the exception for eukaryotic proteins and for many of the peptide hormones.[8] The strategy of mimicry to deceive the natural processes has no less appeal at the exploratory level of molecular design than at the proven level of evolutionary biology.

A chemical strategy for the transformation of 1 to a glycopeptide was identified by the following considerations. First, structural examination of 1 revealed the amino terminus of the peptide as the one point for the introduction of additional functionality. At this location is found the labile (dimethylethoxy)carbonyl group, which is easily removed to expose the free prolyl amine. Second, an N-linkage of the saccharide to the peptide was chosen on the arbitrary assumption that it would provide greater intestinal and metabolic stability than an O-linkage. Third, the saccharide itself was to be from the readily available mono- and disaccharides, rather than the synthetically complex, branched oligosaccharides typical of eukaryotic proteins. These oligosaccharides offer optimal mimicry, but are impractical structural elements for a bulk pharmaceutical. The ultimate choice of a saccharide for the exploration of this concept was 2-(acetylamino)-2-deoxy-D-glucose. This was made with consideration to its prominence in the antennary oligosaccharide structure of the glycoproteins, and particularly with reference to its role as the N-linking saccharide to the amino acid asparagine of the glycoproteins. Lastly, to provide the point of attachment for the saccharide, the Boc-deprotected 1 was lengthened by an additional amino acid. Thus, as the core saccharide–amino acid pair of the endogenous N-linkage is that of *N*-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-N^2-L-asparagine; this pairing was chosen for effecting a glycopeptide guise for ditekiren.

The synthesis of the target glycopeptide 3 was accomplished in a straightforward fashion from the Boc-deprotected 1, by active ester condensation with *N*-[2-(acetylamino)-2-deoxy-3,4,6-triacetyl-β-D-glucopyranosyl]-N^2-[(1,1-dimethylethoxy)carbonyl]-L-asparagine[9] followed by removal of the acetyl protecting groups upon the saccharide of the glycopeptide. In order to provide further comparison, a second saccharide segment (that of β-D-mannopyranose) was introduced to provide the glycopeptide 4, and the advantageous carboxy-terminus *N*-oxide[6] (as found in 2) was provided by peracid conversion of protected 3, ultimately providing 5. The anticipation that the additional amino terminus functionality of 3–5 would not interfere with enzyme binding was confirmed by an IC_{50} in the in vitro human renin inhibition assay (0.4, 0.6, and 0.5 nM, respectively) similar to that of 1 and 2 (0.3 and 0.6 nM, respectively, at pH 6).

Biological evaluation of 3–5 was made with reference to 2, rather than 1, as this latter inhibitor has low aqueous solubility[10] and low oral activity.[4] Oral (5 mg kg^{-1} in 0.1 M citric acid) or intravenous administration (0.07 mg kg^{-1}) of 3–5 in the anesthetized, nephrectomized, ganglion-blocked, human-renin infused rat[11] effected a near maximal blood pressure decrease (greater than 35 mmHg) for the entire duration of this assay (2 h).[12] In contrast, 2 exhibited maximal activity only by the oral route.[6] Thus, on the basis of sustained intravenous hypotensive activity, 3–5 are more potent hypotensive agents than 2 in this animal model. A basis for the improvement of 3–5 after intravenous administration was provided by an evaluation of the disposition properties for inhibitors 2–5. Intravenous or intraduodenal administration to the bile duct cannulated rat permitted the simultaneous comparison of serum concentration of the inhibitor at discrete time

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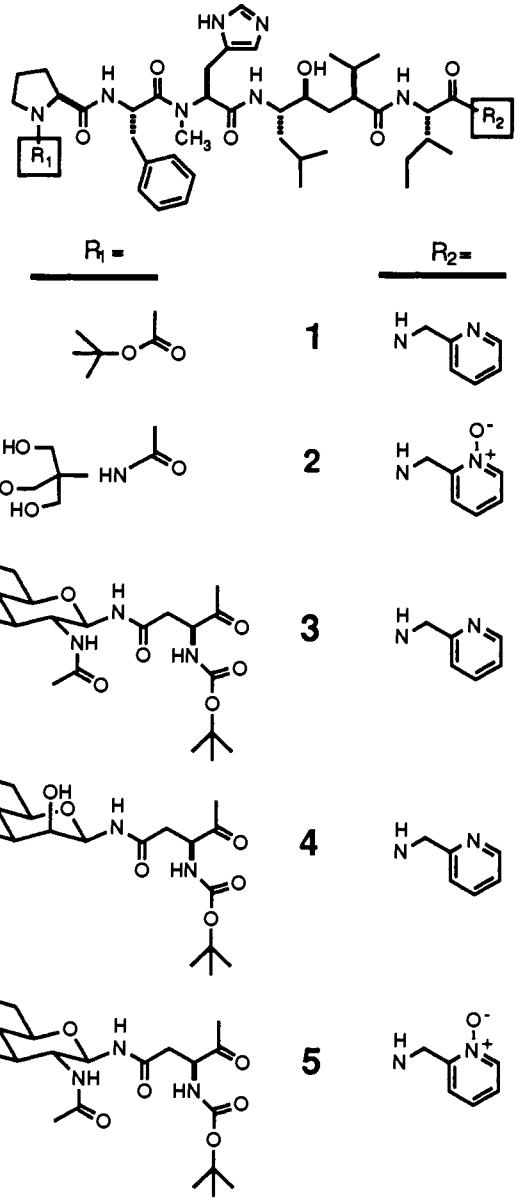
(12) A complete presentation of these data will be provided in the full paper.



points, as well as a comparison of the partition between the bile and urine of the inhibitors.

Intravenous Evaluation. Serum concentration of the inhibitors following injection (1 mg kg^{-1} of **2** and the molar equivalent of this dose for **3–5**) was evaluated at two time points (5 and 60 min) in the bile duct cannulated rat. For all four inhibitors, 5-min serum levels substantially exceeded those found at 60 min (Table I). The glycosylated inhibitors **3–5** gave higher serum levels than seen those seen with **2**. Highest serum concentrations were obtained with **5**. Removal of the *N*-oxide resulted in lower serum levels; substitution of mannose for the *N*-acetylglucosamine lowered serum levels further. Excretion of these inhibitors into the bile was essentially complete after 2 h. The percentage of the dose recovered in the bile appeared to be *inversely* related to the 60-min sera levels. The percentage of the dose recovered in the urine, however, *paralleled* the 60-min sera levels. These observations suggest that the serum levels of these inhibitors are related to the degree with which they evade liver biliary clearance and are available for the less efficient extraction by the kidneys. To further examine this relationship, the log values of the mean serum levels at 60 min were plotted against the percentage of the inhibitor appearing in the bile (Figure 1). The excellent correlation confirms an association

Table I. Serum Concentration and Bile/Urine Partition of 2–5 following Iv Administration to the Bile Duct Cannulated Rat[a]

peptide	serum concentration (nmol/L)		% recovered dose	
			bile[b]	urine[b]
2	1100 ± 130	15 ± 2	83 ± 15	17 ± 2
3	1790 ± 150[c]	20 ± 4	76 ± 5	24 ± 3
4	2190 ± 200[c]	39 ± 4[c]	65 ± 12[c]	35 ± 6[c]
5	2580 ± 120[c]	158 ± 25[c]	33 ± 5[c]	67 ± 6[c]

[a] Mean ± SE. [b] Equal to the biliary (or urinary) recovery, divided by the sum of the biliary and urinary recovery, multiplied by 100%. [c] $P < 0.05$ when compared to **2**.

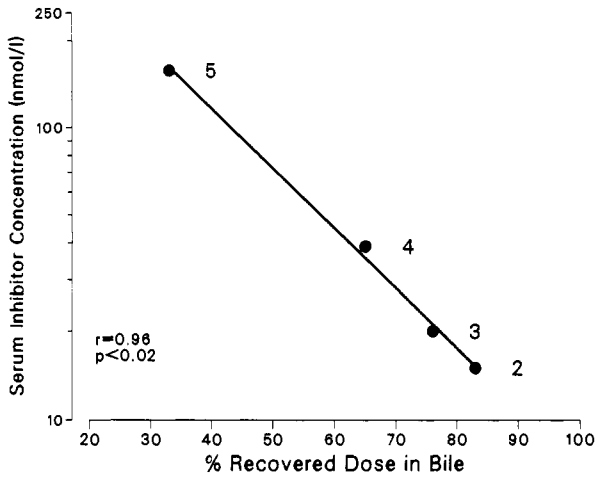


Figure 1. The concentration of the renin inhibitory peptides **2–5** in the serum, 60 min after intravenous administration, versus the percent of the recovered dose in the bile, over the 3-h study period in the bile duct cannulated, anesthetized rat. Peptides (1 mg kg^{-1} of **2** and the molar equivalent of this dose for **3–5**) in a 3 mM citric acid/saline vehicle were injected into the tail vein. Blood was removed from the orbital sinus. The concentration of the inhibitor was determined by a renin enzyme activity assay.[14] Data are means for each group of rats ($n = 4$).

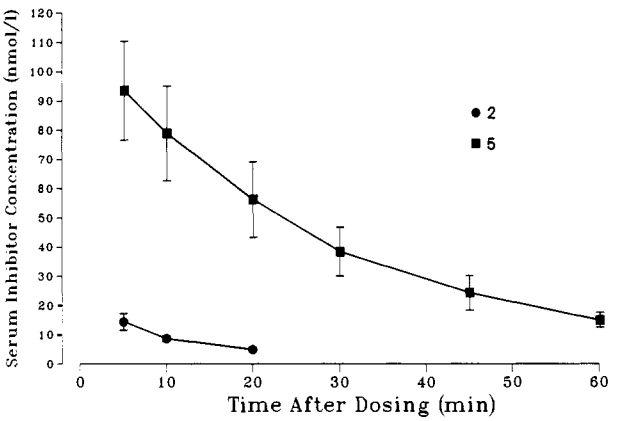


Figure 2. The time course of serum concentration of renin inhibitory peptides **2** and **5** after intraduodenal administration (5 mg kg^{-1} for **2** and the molar equivalent concentration for **5**) to anesthetized rats. The data are means ± standard deviations ($n = 10$).

between the systematic inhibitor levels and the mode of their excretion.

Intraduodenal Evaluation. Intestinal absorption was estimated by comparing the total amount of inhibitor recovered in the bile and urine of the bile duct cannulated rat, after intraduodenal administration (5 mg kg^{-1} for **2** and the molar equivalent of this dose for **3–5**), to the amount recovered after intravenous administration. This comparison gives an estimate of absorption (mean ± SE) for **2** of 8.8 ± 1.9%; **3**, of 8.1 ± 1.2%; **4**, of 4.2 ± 0.4%; and

5, of 7.2 ± 1.8%. Of the four, only the mannose derivative 4 was absorbed to a significantly lesser extent.

A more extensive blood level study after intraduodenal administration of inhibitors 2 and 5 was performed in an additional set of rats without bile duct cannulation. At all time points tested, sera levels of 5 were substantially higher than those of 2, giving a larger area under the curve (Figure 2). Thus, even though the peptides are absorbed to a similar extent, the slower clearance of 5 by the liver results in a much more favorable serum profile.

Conclusions. Transformation of the oligopeptide renin inhibitor 1 to the inhibitor 2 has been observed previously as achieving a noticeable improvement in oral efficacy.[6] In this same report Bundy et al. also describe two 1-[[(2-deoxy-D-glucopyranos-2-C-yl)amino]carbonyl]proline-linked glycopeptide derivatives of 1 with oral hypotensive activity at least equal to that of 2. With the additional glycopeptides 3–5 examined here (of a rather different structural connection to the proline) excellent intravenous and oral hypotensive activity is also observed. *The diversion in the clearance of glycopeptides 3–5 from the rapid and efficient liver biliary pathway to that of the slower renal pathway results in elevated serum concentrations (relative to 2), associated with prolonged hypotensive activity in the human renin infused rat assay.* As these data derive from a limited set of compounds, examined in a single animal species, no broader generalization from this observation is possible. What *is* established, however, is there are to be found defined chemical strategies for the selection of the preferable peptide clearance pathway.[13] Given that the biliary clearance of peptides remains as a significant barrier to the development of therapeutic peptides, it is probable that the value of judicious saccharide–peptide pairing with respect to this issue will receive continuing scrutiny.

Acknowledgment. The laboratories of Donald T. Pals and Don W. DuCharme are thanked for their efforts in securing for 3–5 their IC_{50} values for human renin inhibition and hypotensive evaluation in the human renin infused rat. Suvit Thaisrivongs is thanked for a critical reading of the manuscript.

Supplementary Material Available: Experimental details for the compounds (3 pages). Ordering information is given on any current masthead page.

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