

Renin Inhibitory Pentols Showing Improved Enteral Bioavailability[†]

Heinz-Werner Kleemann,* Holger Heitsch, Rainer Henning, Werner Kramer, Walter Kocher, Ulrich Lerch, Wolfgang Linz, Wolf-Ulrich Nickel, Dieter Ruppert, Hansjörg Urbach, Roland Utz, Adalbert Wagner, Remo Weck, and Freddy Wiegand

Hoechst AG, Postfach 80 03 20, 6230 Frankfurt/M. 80, Germany. Received May 8, 1991

Incorporation of a C-terminal pentahydroxy functionality led to potent, low molecular weight hydrophilic renin inhibitors lacking the P₁' side chain. As these compounds are easy to synthesize and have sufficient water solubility, they were chosen for further study. Compound 33 was transported across rabbit intestinal brush border membrane vesicles and yielded a hypotensive effect in sodium-depleted rhesus monkeys which lasted for 90 min when dosed at 2 mg/kg id.

The aspartic proteinase renin catalyzes the cleavage of angiotensinogen to angiotensin I. This physiologically inactive decapeptide is further processed by angiotensin-converting enzyme to form the potent pressor octapeptide angiotensin II. The major advantage of inhibitors blocking the first step of the system is the unique specificity of renin, offering a selective pharmacological intervention by its inhibitors.

Many renin inhibitors have been shown to lower blood pressure in renin-dependent animal models.¹ The major drawback of most inhibitors is their insufficient oral absorption and the fast hepatic clearance. In order to improve this situation, we focused on decreasing the size and the lipophilicity of the inhibitors.

One class of potent renin inhibitors are amino diols.² Structure-activity investigations³ proved the optimum stereochemistry to be the same as at C-2 and C-3 of readily accessible L-(+)-gulose and D-(+)-mannose. This led us to develop a series of potent renin inhibitors with a hydrophilic sugar-derived C-terminal.

Synthesis

Amino Pentol Derived from L-(+)-Gulose. The synthesis of the protected *gulo*-pentol 6 is outlined in Scheme I. L-(+)-Gulono-1,4-lactone (1) was converted to the diacetone 2⁴ by treatment with 2,2-dimethoxypropane (DMP) containing a catalytic amount of 4-toluenesulfonic acid. Addition of (cyclohexylmethyl)magnesium bromide gave the furanoside 3 as a mixture of the diastereomers, in moderate yield, in addition to some tertiary alcohol 4. Treatment with TiCl₄ and benzylamine⁵ gave the *N*-glycoside 5, which upon reduction with lithium aluminum hydride furnished the protected amino pentol 6 as a 4:1 mixture of diastereoisomers. Attempts to separate the isomers using chromatography failed at this stage, and was performed after acylation with Iva-Phe-Nva-OH. Prior to N-terminal extension, the *N*-benzyl protecting group was removed using ammonium formate in a transfer hydrogenation⁶ to yield *gulo*-pentol 7.

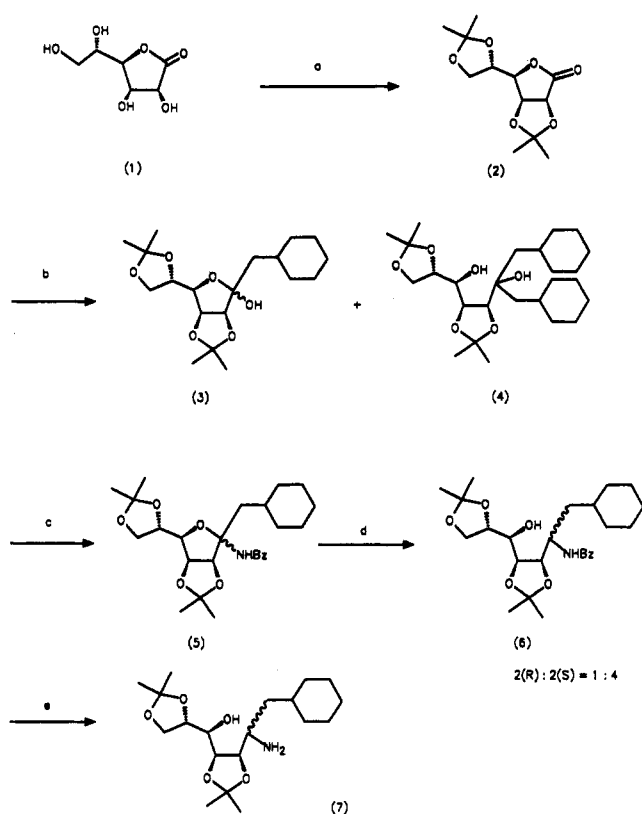
Amino Pentol Derived from D-(+)-Mannose. The synthesis of the protected *manno*-pentol 12 is outlined in Scheme II. D-(+)-Mannose (8) was converted to the *N*-glycosides 10a and 10b in a one-pot reaction sequence via mannofuranoside-diacetonide (9) using benzylamine or α -aminodiphenylmethane. Diastereoselective addition⁷ of (cyclohexylmethyl)lithium produced the protected amino pentols 11a in 95% and 11b in >95% diastereomeric excess. Each of them could be transfer hydrogenated to *manno*-pentol 12.

N-Terminal. 2-Benzyl-3-(*tert*-butylsulfonyl)propionic acid (BBP) was synthesized as described previously.⁸ The

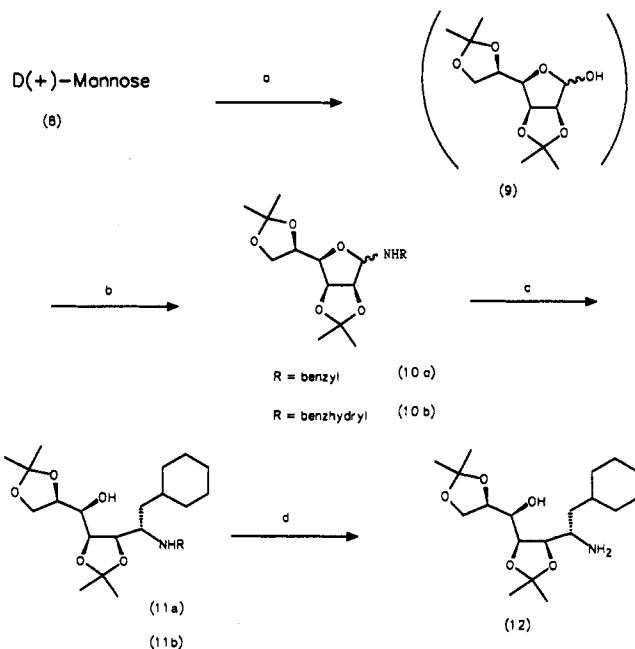
synthesis of 2(S)-[[[N-methyl-N-[2-[N-(morpholino-carbonyl)-N-methylamino]ethyl]amino]carbonyl]oxy]-3-

- (1) Greenlee, W. J. Renin Inhibitors. *Med. Res. Rev.* 1990, 10, 173-236. Bundy, G. L.; Pals, D. T.; Lawson, J. A.; Couch, S. J.; Lipton, M. F.; Mauragis, M. A. Potent Renin Inhibitory Peptides Containing Hydrophilic End Groups. *J. Med. Chem.* 1990, 33, 2276-2283. Rosenberg, S. H.; Woods, K. W.; Sham, H. L.; Kleinert, H. D.; Martin, D. L.; Stein, H.; Cohen, J.; Egan, D. A.; Bopp, B.; Merits, I.; Garren, K. W.; Hoffmann, D. J.; Plattner, J. J. Water-Soluble Renin Inhibitors: Design of a Subnanomolar Inhibitor with a Prolonged Duration of Action. *J. Med. Chem.* 1990, 33, 1962-1969. Henning, R.; Wagner, A.; Schölkens, B. A. Renin Inhibitors. In *Pharmacology of Anti-hypertensive Therapeutics*; Ganten, D., Mulrow, P. J., Eds.; Springer-Verlag: Berlin, 1990; pp 483-505. Breipohl, G.; Geiger, R.; Henke, S.; Kleemann, H.-W.; Knolle, J.; Ruppert, D.; Schölkens, B.; Urbach, H.; Wagner, A.; Wegmann, H. Studies on Renin Inhibitors. In *Topics in Medicinal Chemistry, Fourth SCI-RSC Medicinal Chemistry Symposium*; Leeming, P. R., Ed.; The Royal Society of Chemistry: London, 1988; Special Publication 65, pp 101-127. Bradbury, R. H.; Rivett, J. E. 1,2,4-Triazolo[4,3-a]pyrazine Derivatives with Human Renin Inhibitory Activity. 3. Synthesis and Biological Properties of Aminodeoxystatine and Difluorostatine Derivatives. *J. Med. Chem.* 1991, 34, 151-157. Rosenberg, S. H.; Kleinert, H. D.; Stein, H. H.; Martin, D. L.; Chekal, M. A.; Cohen, J.; Egan, D. A.; Tricarico, K. A.; Baker, W. R. Design of a Well-Absorbed Renin Inhibitor. *J. Med. Chem.* 1991, 34, 469-471. Thaisrivongs, S.; Pals, D. T.; DuCharme, D. W.; Turner, S. R.; DeGraaf, G. L.; Lawson, J. A.; Couch, S. J.; Williams, M. V. Renin Inhibitory Peptides. Incorporation of Polar, Hydrophilic End Groups into an Active Renin Inhibitory Peptide Template and Their Evaluation in a Human Renin Infused Rat Model and in Conscious Sodium-Depleted Monkeys. *J. Med. Chem.* 1991, 34, 633-642. Williams, P. D.; Perlow, D. S.; Payne, L. S.; Holloway, M. K.; Siegl, P. K. S.; Schorn, T. W.; Lynch, R. J.; Doyle, J. J.; Strouse, J. F.; Vlasuk, G. P.; Hoogsteen, K.; Springer, J. P.; Bush, B. L.; Halgren, T. A.; Richards, A. D.; Kay, J.; Veber, D. F. Renin Inhibitors Containing Conformationally Restricted P₁-P₁' Dipeptide Mimetics. *J. Med. Chem.* 1991, 34, 887-900.
- (2) Hanson, G. J.; Baran, J. S.; Lowrie, H. S.; Sarussi, S. J.; Yang, P.-C.; Babler, M.; Bittner, S. E.; Papaioannou, S. E.; Walsh, G. M. Enhanced Potency Dipeptide Glycol Renin Inhibitors: Studies in vitro and in the Conscious Rhesus. *Biochem. Biophys. Res. Commun.* 1987, 146, 959-963. Luly, J. R.; Ba-Maung, N.; Soderquist, J.; Fung, K. L.; Stein, H. H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Dihydroxyethylene Transition-State Mimic at the Scissile Bond To Impart Greater Inhibitory Potency. *J. Med. Chem.* 1988, 31, 2264-2276.
- (3) Kleinert, H. D.; Luly, J. R.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. Renin Inhibitors: Improvement in the stability and biological activity of small peptides containing novel Leu-Val replacements. *FEBS Lett.* 1988, 230, 38-42.
- (4) Ogura, H.; Takahashi, H.; Itoh, T. Reaction of Ethynyl Compounds with Lactones. *J. Org. Chem.* 1972, 37, 72-75.
- (5) White, W. A.; Weingarten, H. A. Versatile New Enamine Synthesis. *J. Org. Chem.* 1967, 32, 213-214.

[†]Dedicated to Professor Dr. Dieter Hoppe on the occasion of his 50th birthday.

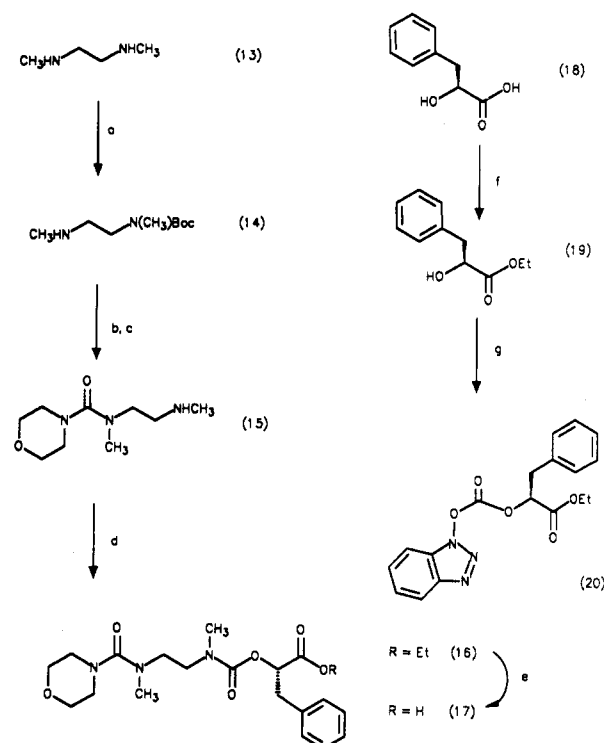
Scheme I^a

^a (a) DMP, *p*-TosOH; (b) (cyclohexylmethyl)magnesium bromide; (c) TiCl₄, benzylamine; (d) LiAlH₄; (e) HCO₂-NH₄⁺, Pd/C.

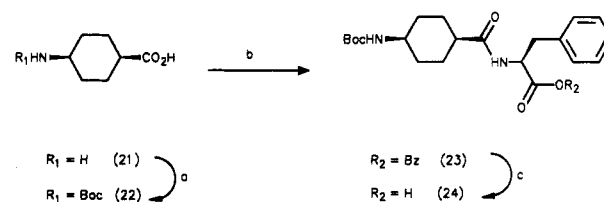
Scheme II^a

^a (a) DMP, *p*-TosOH; (b) R-NH₂; (c) (cyclohexylmethyl)lithium; (d) HCO₂-NH₄⁺, Pd/C.

phenylpropionic acid (MMP) (17) is outlined in Scheme III. *N,N'*-Dimethylethylenediamine (13) was monopro-

Scheme III^a

^a (a) Boc₂O; (b) 1-morpholino-4-(carbonyloxy)benzotriazole; (c) TFA; (d) 20; (e) NaOH; (f) HCl/EtOH; (g) (HOBT)₂CO.

Scheme IV^a

^a (a) Boc₂O; (b) H-Phe-OBz, PPA; (c) H₂, Pd/C.

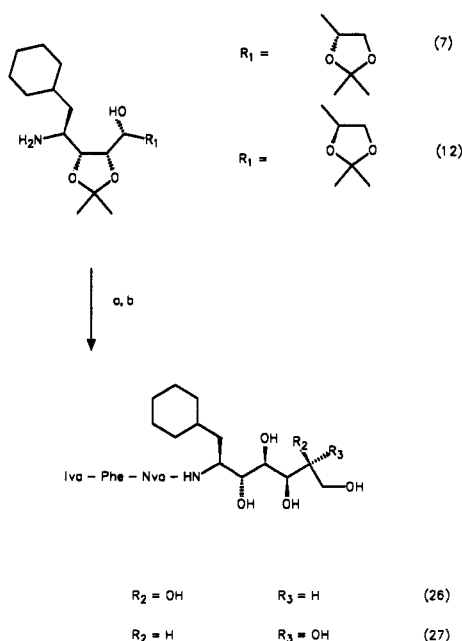
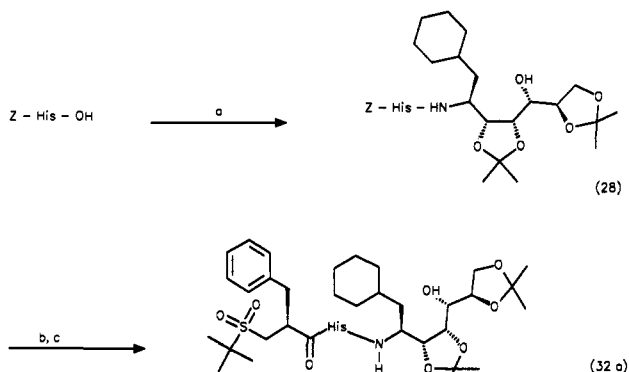
ected to 14 using di-*tert*-butyl dicarbonate (Boc₂O). The unsymmetrical urea 15 was synthesized by sequential treatment of 14 with bis(1-benzotriazolyl) carbonate [(H-OBT)₂CO] and morpholine, followed by deprotection with trifluoroacetic acid (TFA). The completed N-terminal 17 was obtained by reaction of urea 15 with activated carbonate 20 to yield 16 and hydrolysis of the ethyl ester function with NaOH. The synthesis of the carbonate 20 itself proceeded from phenyllactic acid (18) via the ethyl ester (19), which was treated with (HOBT)₂CO to yield 20. Phenylalanine derivative 24 was synthesized from *cis*-4-aminocyclohexanecarboxylic acid (21)⁹ (ACC) (Scheme IV). N-Protection with Boc₂O followed by *n*-propylphosphonic anhydride (PPA) coupling¹⁰ to phenylalanine benzyl ester and subsequent hydrogenation gave N-terminal 24.

(6) Johnstone, R. A. W.; Wilby, A. H.; Entwistle, I. D. Heterogeneous Catalytic Transfer Hydrogenation and Its Relation to Other Methods for Reduction of Organic Compounds. *Chem. Rev.* 1985, 85, 129-170.

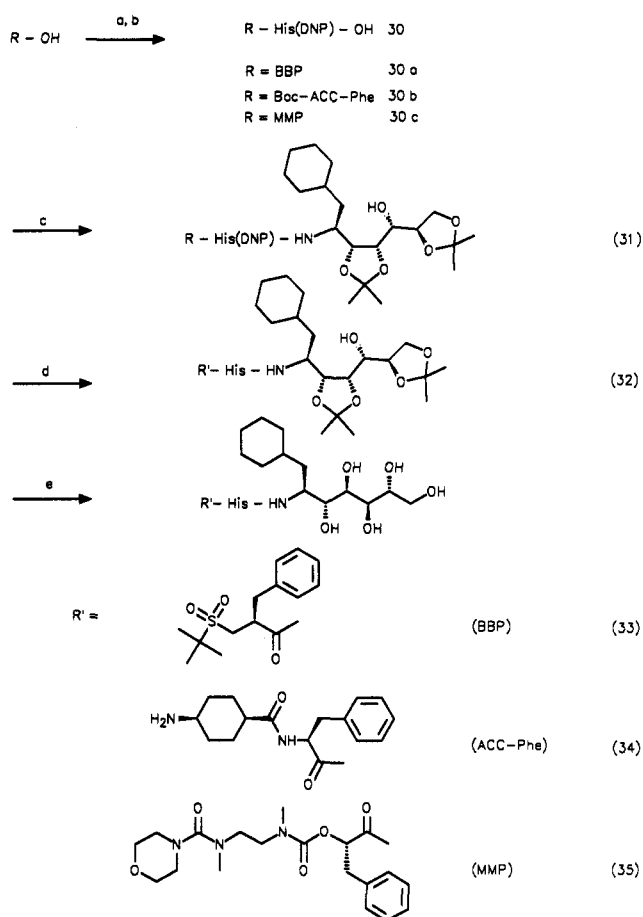
(7) Carcano, M.; Nicotra, F.; Panza, L.; Russo, G. A New Procedure for the Synthesis of D-Glucosamine α -C-Glycosides. *J. Chem. Soc., Chem. Commun.* 1989, 297-298.
 (8) (a) Bühlmyer, P.; Caselli, A.; Fuhrer, W.; Göschke, R.; Rasetti, V.; Rüeger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. Synthesis and Biological Activity of Some Transition-State Inhibitors. *J. Med. Chem.* 1988, 31, 1839-1846. (b) Wagner, A.; Kleemann, H.-W. Hoechst AG, unpublished results.
 (9) Ferber, E.; Brückner, H. *Chem. Ber.* 1943, 10, 1019-1027.
 (10) Wissmann, H.; Kleiner, H.-J. *Angew. Chem.* 1980, 92, 129-130.

Table I. Renin Inhibitory Potency of Pentols

compd	A	B	R ₁	R ₂	IC ₅₀ [nM]	
					kidney	plasma
26	Iva-Phe	Nva	OH	H	120	420
27	Iva-Phe	Nva	H	OH	50	110
33	BBP ^a	His	H	OH	4.4	4.5
34	ACC-Phe ^a	His	H	OH	12	22
35	MMP ^a	His	H	OH	2.4	1.5

^a See Scheme VII.Scheme V^a^a (a) Iva-Phe-Nva-OH (25), pivaloyl chloride, base; (b) *p*-TosOH, methanole.Scheme VI^a^a (a) DPPA; (b) $HCO_2^-NH_4^+$, Pd/C; (c) BBP, HBTU.¹⁵

Completion of the Synthesis. The norvaline (Nva) containing fragment 25 was synthesized using standard peptide coupling methods^{10,11} and attached to the pentols 7 and 12 as outlined in Scheme V. Histidine (His) con-

Scheme VII^a^a (a) DCC, *N*-hydroxysuccinimide; (b) *H*-His(DNP)-OH; (c) pivaloyl chloride, 12; (d) thiophenol; (e) *p*-TosOH or TFA.

taining inhibitors were synthesized by stepwise build-up via an azide coupling¹² of (benzyloxycarbonyl)histidine (*Z*-His-OH) and *manno*-pentol 12 as the crucial reaction to yield the His-amide 28 (Scheme VI). Alternatively, an acylated His fragment 30, with a 2,4-dinitrophenyl (DNP) protecting group¹³ on the imidazole moiety, was coupled

(11) König, W.; Geiger, R. *Chem. Ber.* 1970, 103, 788-798.(12) Shioiri, T.; Ninomiya, K.; Yamada, S. Diphenylphosphoryl Azide. A New Convenient Reagent for a Modified Curtius Reaction and for the Peptide Synthesis. *J. Am. Chem. Soc.* 1972, 94, 6203-6205.(13) Chillemi, F.; Merrifield, R. B. Use of *N*^{im}-Dinitrophenyl-histidine in the Solid-Phase Synthesis of the Tricosapeptides 124-146 of Human Hemoglobin β Chain. *Biochemistry* 1969, 8, 4344-4346.

Table II. Selectivity of Inhibitor 33

aspartic proteinase	IC ₅₀ [M]	aspartic proteinase	IC ₅₀ [M]
pepsin	>10 ⁻⁴	sheep plasma renin	1.0 × 10 ⁻⁷
cathepsin D	>10 ⁻⁴	rhesus monkey plasma renin	1.1 × 10 ⁻⁸
rat plasma renin	8.0 × 10 ⁻⁶	human plasma renin	4.5 × 10 ⁻⁹
dog plasma renin	1.1 × 10 ⁻⁷		

with manno-pentol 12 using pivaloyl chloride¹⁴ as outlined in Scheme VII. Cleavage of the DNP group was performed using thiophenol, followed by acid-catalyzed generation of the pentahydroxy functionality.

Biological Results

In Vitro Activity. The structures and in vitro activities of the renin inhibitors described are shown in Table I. Inhibitor 26 with a gulo stereochemistry C-terminal was significantly less active in both renin assays than the corresponding manno derivative 27. The further structural variations were thus tested with the more easily accessible manno stereochemistry C-terminal. Since BBP-containing inhibitors were shown⁸ to be essentially equipotent to those having Iva-Phe N-terminal, exchange of Nva by His (compound 33) improved potency by approximately 1 order of magnitude in the purified kidney renin assay. The difference is even more pronounced in the physiologically more relevant plasma renin system. Introduction of a N-terminal charged function (compound 34) slightly impaired activity. Introduction of the phenyllactic acid derivative MMP into inhibitor 35 improved activity at the cost of a distinctly enlarged molecular weight. BBP structure 33 was expected to be the best compromise because of its sufficient potency, low molecular weight, chymotrypsin stability (see the Experimental Section), and crystallizability from water. It was therefore selected for in vivo studies.

Selectivity. The renin of species other than primates is only weakly inhibited by compound 33. It did not show any inhibition of pepsin and cathepsin D at concentrations up to 10⁻⁴ M (Table II).

Water Solubility. The solubility of the inhibitors is very dependent on the temperature and the pH of the solution. The selected compound, 33 0.8 g (1.2 mmol), was soluble in 1 L water of 25 °C at pH 7. At 100 °C/pH 7, the solubility rises to 10 g (15 mmol)/L. One liter of water at pH = 4 dissolved at 25 °C 50 g (74 mmol) and, at 100 °C, 100 g (0.15 mol). In conclusion, the renin-inhibiting pentol 33 showed sufficient water solubility to render an enteral formulation possible.

In Vitro Transport Studies. To be intestinally absorbed, drugs must be taken up by small intestinal enterocytes across the brush border membrane. This is the limiting step of intestinal absorption. Small peptides are taken up into small intestine by carrier-mediated processes.^{16,17} Furthermore, two renin inhibitors were shown

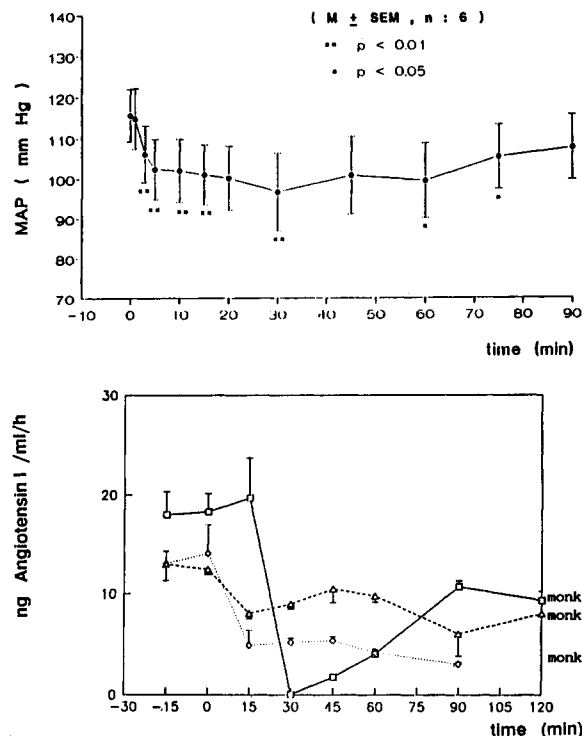


Figure 1. Effects of 2 mg/kg 33 id on MAP and plasma renin activity in sodium-depleted anesthetized rhesus monkeys.

to share this nutrient uptake system.¹⁸ Therefore, we investigated whether pentol 33 also interacts with the proton-dependent uptake system for dipeptides/ β -lactam antibiotics using brush border membrane vesicles prepared from rabbit small intestine. Due to the pentahydroxy moiety, an interaction with the glucose uptake system seemed reasonable. Thus, the effect of 33 on Na⁺/D-glucose cotransport was investigated as well.

The β -lactam antibiotic cephalixin was used as a prototype substrate for the oligopeptide/ β -lactam antibiotic transporter. The H⁺-dependent uptake of 2 mM cephalixin into rabbit small intestine brush border membrane vesicles was inhibited by compound 33 in a concentration-dependent manner with an IC₅₀ of 5–8 mM. On the other hand, the uptake of 33 into brush border membrane vesicles was inhibited by increasing concentrations of cephalixin, suggesting a common transport system for both compounds. A membrane protein of *M*_r 127 000 was identified by photoaffinity labeling techniques as a component of the intestinal dipeptide/ β -lactam antibiotic transporter.^{19,20} The extent of photoaffinity labeling of this protein by [³H]benzylpenicillin was inhibited by 33, demonstrating a direct interaction of the renin inhibitor with the intestinal peptide transport system. The Na⁺-dependent glucose uptake into rabbit small intestinal brush border membrane vesicles was inhibited by 33 in a

- (14) Taschner, E.; Smulkowski, M.; Lubiewska-Nakonieczna, L. *Liebigs Ann.* 1970, 739, 228–230.
- (15) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. O-Benzotriazolyl-N,N,N',N'-tetramethyluronium Hexafluorophosphate as Coupling Reagent for the Synthesis of Peptides of Biological Interest. *Synthesis* 1984, 572–574.
- (16) Mathews, D. M. Intestinal Absorption of Peptides. *Physiol. Rev.* 1975, 537–608.
- (17) Ganapathy, V.; Leibach, F. M. Minireview: Peptide Transport in Intestinal and Renal Brush Border Membrane Vesicles. *Life Sci.* 1982, 30, 2137–2146.

- (18) Kramer, W.; Girbig, F.; Gutjahr, U.; Kleemann, H.-W.; Leipe, I.; Urbach, H.; Wagner, A. Interaction of renin inhibitors with the intestinal uptake system for oligopeptides and β -lactam antibiotics. *Biochem. Biophys. Acta* 1990, 1027, 25–30.
- (19) Kramer, W.; Girbig, F.; Leipe, I.; Petzoldt, E. Direct Photoaffinity Labelling of Binding Proteins for β -Lactam Antibiotics in Rabbit Intestinal Brush Border Membranes with [³H]-Benzylpenicillin. *Biochem. Pharmacol.* 1988, 37, 2427–2435.
- (20) Kramer, W. Identification of identical binding polypeptides for cephalosporins and dipeptides in intestinal brush-border membrane vesicles by photoaffinity labelling. *Biochem. Biophys. Acta* 1987, 905, 65–74.

concentration-dependent manner ($IC_{50} = 3 \text{ mM}$ at a glucose concentration of $20 \mu\text{M}$). However, the uptake of **33** was not inhibited by glucose up to a concentration of 20 mM . These findings indicate that **33** is able to bind to the Na^+ -dependent glucose transporter, but there is no uptake via this carrier. In conclusion, the experimental data demonstrate that renin inhibitory pentol **33** shares the intestinal uptake system for small peptides and β -lactam antibiotics.

In Vivo Activity in Rhesus Monkeys. Compound **33** was given intraduodenally via a gastric fibroscope to anesthetized sodium-depleted rhesus monkeys at a dose of 2 mg/kg . The blood pressure response is shown in Figure 1. It exhibited a rapid onset of action ($<2 \text{ min}$) with a maximum effect of -19 mmHg after 30 min and a duration of about 90 min . The effect was accompanied by a pronounced reduction of plasma renin activity.

Conclusions

A new class of potent hydrophilic renin inhibitors has been found. The lipophilic P_1' side chain (isopropyl in angiotensinogen) was successfully replaced by a pentahydroxy sugar-derived moiety. The selected compound **33** showed nanomolar activity in *in vitro* assays and was transported across brush border membranes of rabbit small intestine. It also exhibited pronounced blood pressure lowering activity after intraduodenal administration in sodium-depleted rhesus monkeys. Finally, it is synthetically easily accessible in some 12% overall yield from mannose.

Experimental Section

Solvents and other reagents were used without further purification unless otherwise noted. Anhydrous THF and diethyl ether were distilled from potassium/benzophenone ketyl. Product solutions were dried over anhydrous Na_2SO_4 (unless otherwise noted) prior to evaporation on a rotary evaporator. Column chromatography was performed on silica gel 60, $0.04\text{--}0.063 \text{ mm}$ (E. Merck), eluting with $5\text{--}20 \text{ psi}$ of pressure. Thin-layer chromatography was done on silica gel 60 plates (E. Merck), and the components were visualized using bis[4-(dimethylamino)-phenyl]methane (TDM) or phosphomolybdic acid reagents. Proton magnetic resonance spectra were measured on a Bruker AM 270. Chemical shifts are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Elemental analyses were performed by the analytical laboratories, Hoechst AG.

1-(Cyclohexylmethyl)gulofuranose 2,3,5,6-Diacetonide (3). A Grignard reagent solution prepared by reaction of 2.33 g (95.8 mmol) of magnesium turnings and 12.0 mL (86.0 mmol) of (bromomethyl)cyclohexane in 200 mL of anhydrous diethyl ether was added dropwise to a refluxing suspension of 20.64 g (80.0 mmol) of lactone **2** in 1.2 L of anhydrous diethyl ether within 3 h . After the addition was completed, the reaction mixture was refluxed for 30 min and then cooled to ambient temperature. Saturated NaHCO_3 solution (400 mL) was added, and the precipitate was removed by filtration. The aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with brine, dried, and evaporated. Chromatography with diisopropyl ether afforded 6.48 g (23%) of the desired product as a white solid, mp $124\text{--}127^\circ\text{C}$ [$\text{TLC } R_f 0.33$]; $[\alpha]_D^{25} 12.4^\circ$ ($c 1.0$, methanol), besides 4.3 g (11%) [$\text{TLC } R_f 0.44$] of **(3R,4R,5R,6S)-1-cyclohexyl-2-(cyclohexylmethyl)-2,3,4,5,6,7-hexahydroxyheptane 3,4,6,7-diacetonide (4)**. **3:** $^1\text{H NMR}$ (CDCl_3) δ $0.95\text{--}1.95$ (m, 13 H), 1.28 (s, 3 H), 1.39 (s, 3 H), 1.44 (s, 6 H), 3.68 (t, 1 H , $J = 8.0 \text{ Hz}$), 4.06 (dd, 1 H , $J^1 = 4.0 \text{ Hz}$, $J^2 = 8.0 \text{ Hz}$), 4.22 (dd, 1 H , $J^1 = 7.0 \text{ Hz}$, $J^2 = 6.0 \text{ Hz}$), 4.30 (m, 1 H), 4.47 (d, 1 H , $J = 6 \text{ Hz}$), 4.71 (dd, 1 H , $J^1 = 4 \text{ Hz}$, $J^2 = 7 \text{ Hz}$). **4:** $^1\text{H NMR}$ (CDCl_3) δ $0.90\text{--}1.10$ (m, 4 H), $1.15\text{--}1.30$ (m, 6 H), 1.32 (s, 3 H), 1.39 (s, 3 H), 1.45 (s, 3 H), 1.58 (s, 3 H), $1.40\text{--}1.85$ (m, 16 H), 1.97 (d, b, 1 H , $J = 13.2 \text{ Hz}$), 3.72 (dd, 1 H , $J^1 = J^2 = 8.0 \text{ Hz}$), $3.88\text{--}3.97$ (m, 3 H), 4.02 (d, 1 H , $J = 6.0 \text{ Hz}$), 4.11 (dd, 1 H , $J^1 = 6.0 \text{ Hz}$, $J^2 = 8.0 \text{ Hz}$), $4.28\text{--}4.38$ (m, 1 H).

N-Benzyl-1-(cyclohexylmethyl)gulofuranosylamine 2,3,5,6-Diacetonide (5). TiCl_4 (1.8 mL , 16.5 mmol) was added to a solution of 6.4 g (18.0 mmol) of furanoside (**3**) and 8.0 mL (72.7 mmol) of benzylamine in 180 mL of anhydrous toluene at 0°C . The mixture was stirred at ambient temperature for 12 h and filtered, 150 mL of water was added, and the mixture was filtered again. The organic phase of the filtrate was separated, washed with water and brine, dried, and evaporated. The residue was chromatographed with diisopropyl ether to yield 4.4 g (55%) of the desired product as a $80:20$ mixture of anomers, $[\alpha]_D^{25} 4.0^\circ$ ($c = 1.0$, methanol). Because of its instability, it was always used immediately after preparation.

(2RS,3R,4R,5R,6S)-2-(Benzylamino)-1-cyclohexyl-3,4,5,6,7-pentahydroxyheptane 3,4,6,7-Diacetonide (6). A solution of 4.4 g (9.9 mmol) of aminoglycoside **5** in 110 mL of anhydrous THF was added dropwise to a solution of 0.38 g (9.9 mmol) of LiAlH_4 in 110 mL of anhydrous THF at 25°C . The resulting reaction mixture was stirred at 25°C for 24 h and quenched by addition of 250 mL of saturated NaHCO_3 solution followed by extraction with ethyl acetate. The organic phase was washed with brine, dried, and evaporated to give 4.3 g (97%) of **6** as a $4:1$ mixture (NMR) of **2S** and **2R** diastereoisomers. The major isomer was assumed to be **2S** because of biological activity.

(2RS,3R,4R,5R,6S)-2-Amino-1-cyclohexyl-3,4,5,6,7-pentahydroxyheptane 3,4,6,7-Diacetonide (7). A 150-mg sample of 10% palladium on charcoal was added to a solution of 740 mg (1.7 mmol) of benzylamine **6** and 1.05 g (0.017 mol) of ammonium formate in 20 mL of methanol. The mixture was refluxed under argon for 90 min . The catalyst was removed by filtration, and the solvent was evaporated. CH_2Cl_2 (50 mL) was added, and the salts were removed by filtration. The filtrate was evaporated to yield 580 mg (98%) of the crude product which was used without further purification.

N-Isovaleryl-Phe-Nva [(2S,3R,4R,5R,6S)-1-Cyclohexyl-3,4,5,6,7-pentahydroxyhept-2-yl]amide (26). Iva-Phe-Nva-OH (580 mg , 1.66 mmol) and 560 mg (1.66 mmol) of **7** were processed using general procedure A. Chromatography with MTB/DIP ($1:1$) yielded 200 mg (18%) of *N*-isovaleryl-Phe-Nva [(2S,3R,4R,5R,6S)-1-cyclohexyl-3,4,5,6,7-di-O-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide as a colorless foam [$\text{TLC } R_f 0.07$], besides 90 mg (8%) of the **2R** product [$\text{TLC } R_f 0.15$]: $^1\text{H NMR}$ (CDCl_3) δ $0.78\text{--}1.05$ (m, 11 H), $1.10\text{--}1.50$ (m, 8 H), $1.60\text{--}1.90$ (m, 6 H), 1.37 (s, 3 H), 1.42 (s, 3 H), 1.44 (s, 3 H), 1.60 (s, 3 H), $1.95\text{--}2.07$ (m, 3 H), 2.82 (d, 1 H , $J = 10.4 \text{ Hz}$), 3.03 (dd, 1 H , $J_1 = 8.4 \text{ Hz}$, $J_2 = 14.0 \text{ Hz}$), 3.18 (dd, 1 H , $J_1 = 6.0 \text{ Hz}$, $J_2 = 14.0 \text{ Hz}$), $3.69\text{--}3.78$ (m, 1 H), 3.95 (dd, 1 H , $J_1 = J_2 = 8.0 \text{ Hz}$), 4.08 (dd, 1 H , $J_1 = J_2 = 8.0 \text{ Hz}$), $4.12\text{--}4.30$ (m, 5 H), $4.66\text{--}4.76$ (m, 1 H), 5.86 (d, 1 H , $J = 8.0 \text{ Hz}$), 6.30 (d, 1 H , $J = 9.2 \text{ Hz}$), 6.78 (d, 1 H , $J = 7.6 \text{ Hz}$), $7.15\text{--}7.34$ (m, 5 H).

A 140-mg (0.20-mmol) portion of the thus obtained diacetonide was deprotected using general procedure B. Chromatography with $\text{CH}_2\text{Cl}_2/\text{methanol}$ ($8:1$) [$\text{TLC } R_f 0.22$] yielded 46 mg (37%) of the desired product as amorphous solid: $^1\text{H NMR}$ ($\text{DMSO}-d_6$) δ 0.67 (d, 3 H , $J = 6.8 \text{ Hz}$), 0.73 (d, 3 H , $J = 6.5 \text{ Hz}$), 0.88 (t, 3 H , $J = 7.2 \text{ Hz}$), $1.10\text{--}1.35$ (m, 8 H), $1.45\text{--}1.90$ (m, 8 H), 2.10 (d, 2 H , $J = 8.5 \text{ Hz}$), $2.95\text{--}3.60$ (m, 6 H), 3.93 (d, 1 H , $J = 7.2 \text{ Hz}$), $4.05\text{--}4.15$ (m, 2 H), $4.20\text{--}4.32$ (m, 1 H), $4.39\text{--}4.45$ (m, 1 H), 4.47 (d, 1 H , $J = 7.3 \text{ Hz}$), $4.50\text{--}4.65$ (m, 3 H), 4.85 (d, 1 H , $J = 7.3 \text{ Hz}$), $7.15\text{--}7.29$ (m, 5 H), 7.40 (d, 1 H , $J = 9.2 \text{ Hz}$), 7.99 (d, 1 H , $J = 8.7 \text{ Hz}$), 8.05 (d, 1 H , $J = 8.0 \text{ Hz}$).

N-Benzylmannofuranosylamine 2,3,5,6-Diacetonide (10a). D-(+)-mannose (10.0 g , 55.5 mmol) and 6.1 mL (55.5 mmol) of benzylamine were processed using general procedure E. Chromatography with diisopropyl ether yielded 15.7 g (81%) of a pale yellow oil. $^1\text{H NMR}$ (CDCl_3) δ 1.34 (s, 3 H), 1.39 (s, 3 H), 1.46 (s, 3 H), 1.49 (s, 3 H), 2.50 (m, 1 H), 3.42 (dd, 1 H , $J^1 = 8.0 \text{ Hz}$, $J^2 = 3.2 \text{ Hz}$), $3.76\text{--}4.00$ (m, 2 H), $4.06\text{--}4.16$ (m, 3 H), $4.38\text{--}4.45$ (m, 1 H), 4.56 (dd, 1 H , $J^1 = 6.4 \text{ Hz}$, $J^2 = 3.2 \text{ Hz}$), 4.68 (dd, 1 H , $J^1 = 6.4 \text{ Hz}$, $J^2 = 3.2 \text{ Hz}$), $7.22\text{--}7.42$ (m, 5 H).

(2S,3R,4R,5R,6R)-2-(Benzylamino)-1-cyclohexyl-3,4,5,6,7-pentahydroxyheptane 3,4,6,7-Diacetonide (11a). *N*-Glycoside **10a** (39.7 g , 0.11 mol), 3.15 g (0.45 mol) of lithium wire ($\Phi = 3.2 \text{ mm}$, high Na), and 40.2 g (0.23 mol) of cyclohexylmethyl bromide were dissolved in 1.1 L of anhydrous tetrahydrofuran and processed in an ultrasonic cleaning bath (Bandelin Sonorex Super RK 1050 BH) at ambient temperature.

After 9 h, the mixture was poured into 1 L of saturated aqueous NaHCO_3 and extracted with 3×500 mL of ethyl acetate. The organic layer was dried and evaporated, and the residue was chromatographed with *tert*-butyl methyl ether/*n*-heptane (1:3) to yield 19.5 g (38%) of the desired product besides 1.1 g (2%) of the 2R compound: ^1H NMR (CDCl_3) δ 0.85–1.10 (m, 2 H), 1.10–1.35 (m, 5 H), 1.37 (s, 6 H), 1.39 (s, 3 H), 1.48 (s, 3 H), 1.50–1.82 (m, 6 H), 2.80 (dd, 1 H, $J^1 = 10.0$ Hz, $J^2 = 3.2$ Hz), 3.28 (d, 1 H, $J = 8.0$ Hz), 3.67 (d, 1 H, $J = 12.4$ Hz), 3.90 (d, 1 H, $J = 12.4$ Hz), 3.95–4.05 (m, 1 H), 4.12–4.20 (m, 3 H), 4.25–4.33 (m, 2 H), 7.23–7.38 (m, 5 H).

N-Benzhydrylmannofuranosylamine 2,3,5,6-Diacetonide (10b). D-(+)-Mannose (300 g, 1.67 mol) and 290 mL (1.67 mol) of benzhydrylamine were processed using general procedure E. Chromatography with *tert*-butyl methyl ether/heptane (1:5) yielded 522 g (74%) of a white solid: mp 82–84 °C; ^1H NMR (CDCl_3) δ 1.38 (s, 3 H), 1.41 (s, 3 H), 1.45 (s, 3 H), 1.48 (s, 3 H), 2.81 (d, b, 1 H, $J = 11.2$ Hz), 3.30 (dd, 1 H, $J^1 = 3.2$ Hz, $J^2 = 8.0$ Hz), 4.05–4.22 (m, 3 H), 4.37–4.45 (m, 1 H), 4.57 (dd, 1 H, $J^1 = 3.2$ Hz, $J^2 = 6.4$ Hz), 4.64 (dd, 1 H, $J^1 = 3.2$ Hz, $J^2 = 6.4$ Hz), 5.57 (s, 1 H), 7.16–7.52 (m, 10 H).

(2S,3R,4R,5R,6R)-2-(Benzhydrylamino)-1-cyclohexyl-3,4,5,6,7-pentahydroxyheptane 3,4,6,7-Diacetonide (11b). Lithium wire (5.2 g) (1% Na) (0.75 mol) was covered with 120 mL of anhydrous diethyl ether under an argon atmosphere. Cyclohexylmethyl bromide (0.42 mL, 3 mmol) was added at room temperature and stirred until the solution turned muddy. The mixture was cooled to –10 °C, and a solution of 42 mL (0.3 mol) of cyclohexylmethyl bromide in 60 mL of anhydrous diethyl ether was added at this temperature. Stirring was continued at –10 °C for 6 h, the mixture was cooled to –30 °C, followed by the addition of a solution of 43 g (0.1 mol) of 10b in 200 mL of anhydrous diethyl ether. The mixture was allowed to warm up to room temperature and stirred for 1 h. Unreacted lithium was removed by filtration, and the filtrate was treated with 1 L of saturated aqueous NaHCO_3 and extracted with 3×500 mL of ethyl acetate. The organic layer was dried and evaporated, and the residue was chromatographed with diisopropyl ether/toluene, 1:5. One obtained 46 g (88%) of the desired product as a colorless oil: ^1H NMR (CDCl_3) δ 0.75–0.95 (m, 2 H), 0.98–1.30 (m, 5 H), 1.29 (s, 3 H), 1.34 (s, 3 H), 1.37 (s, 3 H), 1.54 (s, 3 H), 1.55–1.85 (m, 6 H), 2.72 (d, 1 H, $J = 14.0$ Hz), 3.00–3.07 (m, 1 H), 3.44 (d, 1 H, $J = 7.2$ Hz), 3.93–4.03 (m, 1 H), 4.14–4.22 (m, 3 H), 4.28 (d, 1 H, $J = 8.0$ Hz), 5.04 (s, 1 H), 7.15–7.53 (m, 10 H). (No 2R product was detectable.)

(2S,3R,4R,5R,6R)-2-Amino-1-cyclohexyl-3,4,5,6,7-pentahydroxyheptane 3,4,6,7-Diacetonide (12). To a solution of 0.94 g (1.8 mmol) of 11b in 35 mL of methanol were added 1.13 g (18.0 mmol) of ammonium formate and 190 mg of 10% Pd on charcoal. The mixture was stirred at room temperature for 20 h. The catalyst was removed by filtration, the solvent was evaporated, and the residue was taken up in 50 mL of ethyl acetate. The solution was washed with 50 mL of saturated aqueous NaHCO_3 , and the aqueous layer was extracted with 3×50 mL of ethyl acetate. The organic layer was dried and evaporated. The residue was then chromatographed with MTB to yield 540 mg (85%) of a colorless oil: ^1H NMR (CDCl_3) δ 0.80–1.35 (m, 7 H), 1.38 (s, 3 H), 1.40 (s, 3 H), 1.43 (s, 3 H), 1.53 (s, 3 H), 1.60–1.82 (m, 6 H), 2.95–3.02 (m, 1 H), 3.57 (d, 1 H, $J = 8.0$ Hz), 3.99–4.08 (m, 1 H), 4.12–4.20 (m, 3 H), 4.42 (d, 1 H, $J = 8.0$ Hz).

N-Isovaleroyl-Phe-Nva [(2S,3R,4R,5R,6R)-1-Cyclohexyl-3,4,5,6,7-pentahydroxyhept-2-yl]amide (27). Protected amino pentol 12 (194 mg, 0.54 mmol) and 189 mg (0.54 mmol) of Iva-Phe-Nva-OH were coupled using general procedure A. Chromatography with MTB/DIP (1:1) [TLC R_f 0.25] yielded 62 mg (17%) of N-isovaleroyl-Phe-Nva [(2S,3R,4R,5R,6R)-1-cyclohexyl-3,4,5,6,7-di-O-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide as a colorless foam: ^1H NMR (CDCl_3) δ 0.80–1.05 (m, 11 H), 1.10–2.10 (m, 14 H), 1.27 (s, 3 H), 1.34 (s, 3 H), 1.39 (s, 3 H), 1.43 (s, 3 H), 3.03 (dd, 1 H, $J^1 = 8.4$ Hz, $J^2 = 14.0$ Hz), 3.17 (dd, 1 H, $J^1 = 5.6$ Hz, $J^2 = 14.0$ Hz), 3.52–3.60 (m, 1 H), 3.90–4.05 (m, 2 H), 4.10–4.38 (m, 5 H), 4.65–4.75 (m, 1 H), 5.83 (d, 1 H, $J = 7.2$ Hz), 6.21 (d, 1 H, $J = 8.8$ Hz), 6.80 (d, 1 H, $J = 7.2$ Hz), 7.15–7.35 (m, 5 H). The thus obtained diacetonide (62 mg, 90 μmol) was deprotected using general procedure B. Chromatography with CH_2Cl_2 /methanol (8:1) [TLC R_f 0.20] yielded 21 mg

(38%) of the pentol 27 as amorphous solid: ^1H NMR ($\text{DMSO}-d_6/\text{D}_2\text{O}$) δ 0.67 (d, 3 H, $J = 6.4$ Hz), 0.74 (d, 3 H, $J = 6.4$ Hz), 0.87 (t, 3 H, $J = 7.2$ Hz), 0.80–1.00 (m, 2 H), 1.10–1.35 (m, 8 H), 1.45–1.92 (m, 10 H), 2.73 (dd, 1 H, $J^1 = 10.8$ Hz, $J^2 = 14.0$ Hz), 3.01 (dd, 1 H, $J^1 = 4.0$ Hz, $J^2 = 14.0$ Hz), 3.33–3.65 (m, 6 H), 4.08–4.17 (m, 1 H), 4.24–4.31 (m, 1 H), 4.52–4.59 (m, 1 H), 7.15–7.28 (m, 5 H).

[2-Benzyl-3-(*tert*-butylsulfonyl)propionyl]-N^{im}-(2,4-dinitrophenyl)histidine (30a). Boc-His(DNP)-OH¹⁸ (6.5 g, 15.4 mmol) and 5.4 g (14.1 mmol) of 2-benzyl-3-(*tert*-butylsulfonyl)propionic acid hydroxysuccinimide ester were processed using general procedure D to yield 6.1 g (75%) of a yellow solid: ^1H NMR (CDCl_3) δ 1.18 (s, 9 H), 2.60–2.82 (m, 2 H), 3.02–3.15 (m, 1 H), 3.30–3.65 (m, 4 H), 4.75–4.85 (m, 1 H), 7.12–7.27 (m, 5 H), 7.68 (s, 1 H), 8.26 (d, 1 H, $J = 8.0$ Hz), 8.53 (dd, 1 H, $J^1 = 1.8$ Hz, $J^2 = 8.0$ Hz), 8.88 (d, 1 H, $J = 1.8$ Hz), 9.02 (s, b, 1 H).

[2-Benzyl-3-(*tert*-butylsulfonyl)propionyl]-N^{im}-(2,4-dinitrophenyl)histidyl [(2S,3R,4R,5R,6R)-1-Cyclohexyl-3,4,5,6,7-di-O-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide (31a). The N-terminal fragment 30a (1.32 g, 2.24 mmol) and 0.28 mL (2.24 mmol) of the C-terminal fragment 12 were coupled using the general procedure A; 1.45 g of a yellow foam [TLC R_f 0.62 (ethyl acetate/methanol, 5:1)] was obtained, which was further processed without purification.

[2-Benzyl-3-(*tert*-butylsulfonyl)propionyl]histidyl [(2S,3R,4R,5R,6R)-1-Cyclohexyl-3,4,5,6,7-di-O-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide (32a). Crude 31a (1.45 g) was deprotected using general procedure C. Chromatography with toluene/methanol (5:1) yielded 400 mg (47%) of the desired product as white crystals: mp 160 °C; ^1H NMR δ 0.80–1.00 (m, 2 H), 1.10–1.30 (m, 5 H), 1.36 (s, 15 H), 1.41 (s, 6 H), 1.55–1.88 (m, 6 H), 2.78–3.25 (m, 6 H), 3.57–3.63 (m, 2 H), 3.90–4.35 (m, 7 H), 4.53–4.60 (m, 1 H), 6.22 (d, 1 H, $J = 8.0$ Hz), 6.82 (d, 1 H, $J = 7.6$ Hz), 7.08 (s, 1 H), 7.15–7.32 (m, 6 H), 7.48 (s, 1 H).

[2-Benzyl-3-(*tert*-butylsulfonyl)propionyl]histidyl [(2S,3R,4R,5R,6R)-1-Cyclohexyl-3,4,5,6,7-pentahydroxyhept-2-yl]amide (33). Bisacetonide 32a (380 mg, 0.5 mmol) was deprotected using the general procedure B. Crystallization from water yielded 150 mg (44%) colorless needles: mp 186 °C; ^1H NMR δ 0.70–0.97 (m, 2 H), 1.10–1.32 (m, 5 H), 1.40–1.85 (m, 6 H), 1.19 (s, 9 H), 2.55–3.07 (m, 5 H), 3.18–3.66 (m, 10 H), 3.88–3.95 (m, 1 H), 4.10–4.20 (m, 1 H), 4.25–4.40 (m, 1 H), 4.45–4.55 (m, 1 H), 4.58–4.90 (m, 1 H), 6.87 (s, 1 H), 7.18–7.40 (m, 7 H), 7.53 (s, 1 H), 8.37 (d, 1 H, $J = 7.2$ Hz).

N- α -(Benzyloxycarbonyl)histidyl [(2S,3S,4R,5R,6R)-1-Cyclohexyl-3,4,5,6,7-di-O-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide (28). Amino pentol diacetonide 12 (6.4 g, 17.8 mmol) and 5.1 g (17.8 mmol) of N- α -(benzyloxycarbonyl)histidine were dissolved in 100 mL of DMF. A solution of 4.9 g (17.8 mmol) of diphenyl phosphorazidate in 15 mL of DMF was added at 0 °C followed by 2.9 mL (16.8 mmol) of ethyldiisopropylamine in 10 mL of DMF. Stirring was continued at 0 °C for 18 h and at room temperature for 5 days. The mixture was diluted with 1 L of ethyl acetate, washed twice with 100 mL of 0.7 M aqueous KH_2PO_4 and twice with 100 mL of 5% aqueous Na_2CO_3 , dried, and evaporated. The residue was dissolved in 20 mL of ethyl acetate and the crude product precipitated with 200 mL of *n*-heptane. The precipitate was extracted with 200 mL of diisopropyl ether and filtrated. The diisopropyl ether solution was evaporated to yield 5.9 g (50%) of the desired product [TLC R_f 0.27 (ethyl acetate/methanol, 10:1)]: ^1H NMR (CDCl_3) δ 0.73–1.00 (m, 2 H), 1.03–1.30 (m, 5 H), 1.36 (s, 3 H), 1.38 (s, 3 H), 1.45 (s, 6 H), 1.55–1.90 (m, 6 H), 3.03 (dd, 1 H, $J^1 = 5.2$ Hz, $J^2 = 15.2$ Hz), 3.18 (dd, 1 H, $J^1 = 4.0$ Hz, $J^2 = 15.2$ Hz), 3.60–3.70 (m, 1 H), 3.88–4.00 (m, 2 H), 4.12–4.30 (m, 4 H), 4.32–4.42 (m, 1 H), 5.03 (d, 1 H, $J = 12.0$ Hz), 5.10 (d, b, 1 H), 5.18 (d, 1 H, $J = 12.0$ Hz), 6.43 (d, 1 H, $J = 8.4$ Hz), 6.81 (s, 1 H), 7.30–7.40 (m, 5 H), 7.52 (s, 1 H), 9.33 (s, b, 1 H).

Alternative Procedure To Prepare [2-Benzyl-3-(*tert*-butylsulfonyl)propionyl]histidyl [(2S,3R,4R,5R,6R)-1-Cyclohexyl-3,4,5,6,7-di-O-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide (32a). Z-protected compound 28 (4.6 g, 7.3 mmol) and 4.6 g (73 mmol) of ammonium formate were dissolved in 100 mL of methanol, and 3 g of 10% Pd on charcoal was added. The mixture was stirred at room temperature for 4

h, filtrated, and evaporated. The residue was dissolved using 500 mL of ethyl acetate and washed with 50 mL of 5% aqueous Na_2CO_3 , 100 mL of water, and 100 mL of saturated aqueous NaCl. Evaporation yielded 3.6 g (100%) of histidyl [(2*S*,3*S*,4*R*,5*R*,6*R*)-1-cyclohexyl-3,4,6,7-di-*O*-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide which was used without further purification.

To a solution of 0.79 g (2.8 mmol) of 2-Benzyl-3-(*tert*-butylsulfonyl)propionic acid in 20 mL of DMF were added 0.38 mL (2.8 mmol) of triethylamine and 1.1 g (2.8 mmol) of HBU. The mixture was stirred for 5 min, and a solution of 1.6 g (2.8 mmol) of the above His-pentol compound in 15 mL of DMF was added. Stirring was continued for 21 h at room temperature, and the solvent was evaporated. The residue was dissolved in 500 mL of ethyl acetate and washed with 3×100 mL of 5% aqueous Na_2CO_3 . The organic layer was dried and evaporated. The residue was chromatographed with CH_2Cl_2 /methanol (10:1) to yield 700 mg (33%) of the desired product.

***N*-(*tert*-Butoxycarbonyl)-*N*-methyl-2-(methylamino)ethylamine (14).** A solution of 12.5 g (55 mmol) of di-*tert*-butyl dicarbonate in 25 mL of CH_2Cl_2 was added dropwise to 100 g of *N,N*-dimethylethylenediamine (13) at 5 °C. The mixture was stirred at room temperature for 4 h and then evaporated. The residue was dissolved in 100 mL of ethyl acetate and washed with 100 mL of saturated aqueous Na_2CO_3 and 100 mL of saturated aqueous NaCl. The organic layer was dried and evaporated to yield 9.3 g (90%) of the crude product which was used without further purification: ^1H NMR (CDCl_3) δ 1.45 (s, 9 H), 2.42 (s, 3 H), 2.50–2.85 (m, 3 H), 2.86 (s, 3 H), 3.33 (t, 2 H, J = 6.6 Hz).

***N*-Methyl-2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethylamine (15).** Twenty-one grams (21 mmol) of 70% bis-(hydroxybenzotriazolyl) carbonate and 4.0 mL (50 mmol) of pyridine were dissolved in 210 mL of CH_2Cl_2 , and 4.4 mL (50 mmol) of morpholine in 50 mL of CH_2Cl_2 was added dropwise at room temperature. The resulting clear solution was stirred at this temperature for 4 h, followed by addition of 9.4 g (50 mmol) of 14 in 90 mL of CH_2Cl_2 . Stirring was continued for 48 h, and the mixture was washed with 100 mL of saturated aqueous Na_2CO_3 , 100 mL of 5% aqueous NaHSO_4 , and 100 mL of brine. The organic layer was dried and evaporated. Chromatography with ethyl acetate [TLC R_f 0.25] yielded 4.6 g (31%) of *N*-*tert*-butoxycarbonyl)-*N*-methyl-2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethylamine: mp 88 °C; ^1H NMR (CDCl_3) δ 1.45 (s, 9 H), 2.87 (s, 3 H), 2.93 (s, 3 H), 3.10–3.35 (m, 4 H), 3.36 (s, 4 H), 3.55–3.83 (m, 4 H). This Boc compound was deprotected as follows: 350 mg (1.2 mmol) were dissolved in 25 mL of 6 N HCl in dimethoxyethane and stirred at room temperature for 3 h. The reaction mixture was evaporated, and the crude 15 was used without purification.

Ethyl 2(*S*)-[[[*N*-Methyl-*N*-[2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethyl]amino]carbonyl]oxy]-3-phenylpropionate (16). Ethyl 2(*S*)-hydroxy-3-phenylpropionate (0.19 g, 1.0 mmol) in 6 mL of CH_2Cl_2 was added dropwise to 0.43 g (1.0 mmol) of 70% bis(hydroxybenzotriazolyl) carbonate and 82 μL (1.0 mmol) of pyridine in 10 mL of CH_2Cl_2 at room temperature. Stirring was continued for 5 h, followed by addition of 82 μL (1.0 mmol) of pyridine. Finally, a solution of the crude 15 in 5 mL of CH_2Cl_2 was added, and the mixture was stirred at room temperature for 18 h. The solution was washed with 50 mL of saturated aqueous Na_2CO_3 , 50 mL of 5% aqueous NaHSO_4 , and 50 mL of brine, dried, and evaporated. Chromatography with ethyl acetate yielded 150 mg (38%) of an amorphous solid. [TLC R_f 0.19]: ^1H NMR (CDCl_3) δ 1.25 (t, 3 H, J = 7.0 Hz), 2.86 (s, b, 3 H), 2.93 (s, 3 H), 3.00–3.30 (m, 6 H), 3.38 (s, b, 4 H), 3.50–3.83 (m, 4 H), 4.22 (q, 2 H, J = 7.0 Hz), 4.92–5.30 (m, 1 H), 7.30 (s, 5 H).

2(*S*)-[[[*N*-Methyl-*N*-[2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethyl]amino]carbonyl]oxy]-3-phenylpropionic Acid (17). Ester 16 (150 mg, 0.36 mmol) was dissolved in 10 mL of ethanol, and 3.6 mL of 0.1 N aqueous NaOH was added at room temperature. The mixture was stirred for 2 h, the ethanol was evaporated, aqueous HCl was added to pH 2, and the mixture was extracted three times with 20 mL of ethyl acetate. The organic layer was dried and evaporated to yield 135 mg (95%) of an amorphous solid which was used without further purification: ^1H NMR (CDCl_3) δ 2.90 (s, b, 6 H), 3.03–3.50 (m, 8 H), 3.50–3.93 (m,

6 H), 5.00–5.47 (m, 1 H), 7.30 (s, 5 H).

2(*S*)-[[[*N*-Methyl-*N*-[2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethyl]amino]carbonyl]oxy]-3-phenylpropionyl]-*N*^{im}-(2,4-dinitrophenyl)histidine (30c). Boc-His(DNP)-OH¹⁶ (650 mg, 1.5 mmol) and 750 mg (1.5 mmol) of 2(*S*)-[[[*N*-methyl-*N*-[2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethyl]amino]carbonyl]oxy]-3-phenylpropionic acid hydroxysuccinimide ester were processed using general procedure D to yield 460 mg (43%) of a yellow amorphous solid: ^1H NMR (CDCl_3) δ 2.50–3.65 (m, 22 H), 4.68–4.82 (m, 1 H), 4.94–5.03 (m, 1 H), 7.05–7.30 (m, 6 H), 7.52 (s, 1 H), 8.15–8.25 (2 d, 1 H), 8.55–8.67 (2 d, 1 H), 8.88 (s, 1 H), 9.10 and 9.14 (2 s, 1 H) (2 rotamers).

2(*S*)-[[[*N*-Methyl-*N*-[2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethyl]amino]carbonyl]oxy]-3-phenylpropionyl]histidyl [(2*S*,3*R*,4*R*,5*R*,6*R*)-1-Cyclohexyl-3,4,6,7-di-*O*-isopropylidene 3,4,5,6,7-pentahydroxyhept-2-yl]amide (32c). N-Terminal fragment 30c (450 mg, 0.65 mmol) and 230 mg (0.65 mmol) of C-terminal diacetone 12 were coupled via general procedure A. The crude product was then subjected to *N*^{im}-deprotection following general procedure C. Chromatography with ethyl acetate/methanol (5:1) [TLC R_f 0.18] yielded 29 mg of a colorless foam: ^1H NMR (CDCl_3) δ 0.80–1.00 (m, 2 H), 1.10–1.35 (m, 5 H), 1.27 (s, 6 H), 1.33 and 1.37 (2 s, 3 H), 1.41 and 1.43 (2 s, 3 H), 1.50–1.70 (m, 6 H), 2.77–2.95 (m, 6 H), 3.05–3.20 (m, 8 H), 3.25–3.45 (m, 4 H), 3.55–3.70 (m, 4 H), 3.90–4.08 (m, 3 H), 4.10–4.32 (m, 4 H), 4.50–4.60 (m, 1 H), 5.13–5.20 and 5.22–5.28 (2 m, 1 H), 6.52–6.60 (m, 1 H), 6.63–6.70 (m, 1 H), 6.74–6.80 (m, 1 H), 7.17–7.33 (m, 6 H), 7.50–7.60 (m, 1 H) (2 rotamers).

2(*S*)-[[[*N*-Methyl-*N*-[2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethyl]amino]carbonyl]oxy]-3-phenylpropionyl]histidyl [(2*S*,3*R*,4*R*,5*R*,6*R*)-1-Cyclohexyl-3,4,5,6,7-pentahydroxyhept-2-yl]amide (35). Diacetone 31c (28 mg, 32 μmol) was dissolved in 2 mL of CH_2Cl_2 , and 2 mL of trifluoroacetic acid was added. The solution was stirred at room temperature for 3 h, poured into 50 mL of saturated aqueous Na_2CO_3 , and extracted with 3×50 mL of ethyl acetate. The organic layer was dried and evaporated. Chromatography with ethyl acetate/methanol (1:1) [TLC R_f 0.22] yielded 13 mg (51%) of an amorphous solid: ^1H NMR (CDCl_3) δ 0.65–0.92 (m, 2 H), 1.00–1.20 (m, 5 H), 1.45–1.75 (m, 6 H), 2.70–3.95 (m, 33 H), 4.15–4.30 (m, 1 H), 4.65–4.77 (m, 1 H), 5.00–5.13 (m, 1 H), 6.72 and 6.78 (2 s, 1 H), 7.10–7.33 (m, 7 H), 7.60–7.68 (s, b, 1 H), 7.95–8.05 and 8.13–8.27 (2 m, 1 H) (2 rotamers).

***N*-(*tert*-Butoxycarbonyl)-*cis*-4-aminocyclohexanecarboxylic Acid (22).** NaOH (1.5 g, 38 mmol) and 5.0 g (35 mmol) of ACC 21 were dissolved in 40 mL of water, 45 mL of *tert*-butyl alcohol and 7.6 g (35 mmol) of di-*tert*-butyl dicarbonate were added, and the mixture was stirred for 1 h at room temperature. The solution was washed with 3×50 mL of *n*-hexane, 50 mL of 1 N aqueous HCl was added, and the mixture was extracted with 3×50 mL of ethyl acetate. The organic layer was dried and evaporated to yield 6.6 g (78%) of a white crystalline solid: mp 164–168 °C; ^1H NMR (CDCl_3) δ 1.48 (s, 9 H), 1.75 (m, 8 H), 2.52 (m, 1 H), 3.60 (m, 1 H).

***N*-[[*N*-(*tert*-Butoxycarbonyl)-*cis*-4-aminocyclohexyl]carbonyl]phenylalanine Benzyl Ester (23).** 22 (6.0 g, 25 mmol) and 6.3 g (25 mmol) of phenylalanine benzyl ester were dissolved in 75 mL of DMF, the mixture was cooled to 0 °C, and 15.7 mL (123 mmol) of *N*-ethylmorpholine and 24 mL 50% (weight) of *n*-propylphosphonic anhydride (PPA) in CH_2Cl_2 was added. The mixture was stirred for 18 h, 100 mL of CH_2Cl_2 was added, and the mixture was washed with 100 mL of saturated aqueous NaHCO_3 and 100 mL of 5% aqueous NaHSO_4 . The organic layer was dried and evaporated, and the residue was chromatographed with cyclohexane/ethyl acetate (1:1) [TLC R_f 0.47]: yield 11.1 g (93%) colorless oil; ^1H NMR (CDCl_3) δ 1.45 (s, 9 H), 1.65 (m, 8 H), 2.17 (m, 1 H), 3.08 (dd, 1 H, J^1 = 13.6 Hz, J^2 = 6.4 Hz), 3.18 (dd, J^1 = 13.6 Hz, J^2 = 6.0 Hz), 3.69 (m, 1 H), 4.60 (d, 1 H, J = 8.0 Hz), 4.90–4.98 (m, 1 H), 5.12 (d, 1 H, J = 12.0 Hz), 5.21 (d, 1 H, J = 12.0 Hz), 5.88 (d, 1 H, J = 8.2 Hz), 6.98 (m, 2 H), 7.21 (m, 3 H), 7.3 (m, 5 H).

***N*-[[*N*-(*tert*-Butoxycarbonyl)-*cis*-4-aminocyclohexyl]carbonyl]phenylalanine (24).** 23 (5.5 g, 11.4 mmol) was dissolved in 230 mL of ethanol and hydrogenated for 4 h under

atmospheric pressure using 1.1 g 10% Pd/C. The catalyst was removed by filtration and the ethanol evaporated. The residue was crystallized from ethyl acetate to yield 4.1 g (92%) of the desired product: mp 160–161 °C; ^1H NMR (CDCl_3) δ 1.49 (s, 9 H), 1.66 (m, 1 H), 3.1 (m, 2 H), 3.7 (m, 1 H), 4.9 (d, 1 H), 6.9 (d, 1 H), 7.3 (m, 5 H).

[N-[[N-(*tert*-Butoxycarbonyl)-*cis*-4-aminocyclohexyl]-carbonyl]phenylalanyl]-*N*^{im}-(2,4-dinitrophenyl)histidine (30b). Boc-His(DNP)-OH (1.1 g, 2.6 mmol) and 1.0 g (2.1 mmol) were processed using general procedure D. Chromatography with CH_2Cl_2 /methanol (10:1) [TLC R_f 0.08] yielded 0.86 g (61%) of a yellow foam which was used without further purification.

[N-[[N-(*tert*-Butoxycarbonyl)-*cis*-4-aminocyclohexyl]-carbonyl]phenylalanyl]histidyl [(2*S*,3*R*,4*R*,5*R*,6*R*)-1-Cyclohexyl-3,4,6,7-di-*O*-isopropylidene-3,4,5,6,7-penta-hydroxyhept-2-yl]amide (32b). 30b (0.86 g, 1.2 mmol) and 0.41 g (1.1 mmol) of 12 were coupled using general procedure A, and the *N*^{im} DNP-protection was removed from the crude product via general procedure C. Chromatography with ethyl acetate/methanol (5:1) [TLC R_f 0.41] yielded 0.40 g (40%) of a colorless foam: ^1H NMR (CDCl_3) δ 0.70–1.00 (m, 2 H), 1.03–1.30 (m, 5 H), 1.36 (s, 3 H), 1.40 (s, 3 H), 1.42 (s, 3 H), 1.47 (s, 12 H), 1.50–1.75 (m, 14 H), 2.15–2.30 (m, 1 H), 3.00–3.20 (m, 3 H), 3.53–3.62 (m, 2 H), 3.63–3.74 (m, 1 H), 3.90–4.40 (m, 7 H), 4.60–4.77 (m, 2 H), 4.88–4.97 (m, 1 H), 6.10–6.22 (m, 1 H), 6.45–6.55 (m, 1 H), 6.70–6.77 (m, 1 H), 7.08–7.55 (m, 8 H).

[N-[(*cis*-4-Aminocyclohexyl)carbonyl]phenylalanyl]-histidyl [(2*S*,3*R*,4*R*,5*R*,6*R*)-1-Cyclohexyl-3,4,5,6,7-penta-hydroxyhept-2-yl]amide (34). 32b (180 mg, 0.21 mmol) was dissolved in 5 mL of CH_2Cl_2 , and 5 mL of trifluoroacetic acid was added at 0 °C. The mixture was stirred at this temperature for 7 h, poured into 100 mL of saturated aqueous Na_2CO_3 , and extracted with 3 \times 100 mL of ethyl acetate. The organic layer was dried and evaporated. Chromatography of the residue with acetone/water/saturated aqueous NH_3 (100:10:5) [TLC R_f 0.12] yielded 34 mg (24%) of a colorless foam: ^1H NMR ($\text{DMSO}-d_6$) δ 0.70–1.00 (m, 2 H), 1.04–1.35 (m, 5 H), 1.50–1.71 (m, 14 H), 2.16–2.25 (m, 1 H), 2.70–3.10 (m, 4 H), 3.15–3.65 (m, 13 H), 3.90–4.03 (m, 1 H), 4.08–4.20 (m, 1 H), 4.40–4.52 (m, 2 H), 4.68–4.80 (m, 2 H), 6.82 (s, 1 H), 7.12–7.29 (m, 5 H), 7.36 (d, 1 H, $J = 9.2$ Hz), 7.51 (s, 1 H), 7.92–8.02 (m, 1 H), 8.15–8.25 (m, 1 H).

General Procedures. The following experimental procedures provide representative conditions for the preparation of all compounds shown in the tables.

A. Mixed Anhydride Coupling Using Pivaloyl Chloride.¹⁴ The carboxylic acid representing the N-terminal fragment (1.6 mmol), 0.22 mL (1.6 mmol) of *N*-ethylpiperidine, and 0.23 mL of Et_3N were dissolved in 25 mL of anhydrous CH_2Cl_2 . Pivaloyl chloride (0.20 mL) was added at –15 °C and converted to the mixed anhydride by stirring at room temperature for 10 min. The mixture was cooled to –10 °C, and a solution of 1.6 mmol of the amino fragment in 10 mL of anhydrous CH_2Cl_2 was added. Stirring was continued at room temperature for 20 h, and the solvent was evaporated. The residue was dissolved in 150 mL of ethyl acetate, washed two times (50 mL of 0.6 M KH_2PO_4 , 50 mL of saturated aqueous NaHCO_3), dried, and chromatographed to homogeneity.

B. Removal of Acetonide Protection. Nonbasic diacetonides were dissolved in 50 mL/mmol of methanol and 2 mL/mmol of water, and a catalytic amount of 4-toluenesulfonic acid was added. Histidine-containing diacetonides required 1.1 molar equiv of the acid. The mixture was stirred at room temperature for 3 days. Volatiles were evaporated, and the residue was chromatographed to homogeneity.

C. Removal of *N*^{im}-(2,4-Dinitrophenyl) Protection. The *N*^{im}-(2,4-dinitrophenyl)histidyl derivative (1 mmol) and 8 equiv of thiophenol were dissolved in 15 mL of acetonitrile, and the mixture was stirred at room temperature for 2 h. The volatiles were evaporated, and the residue was chromatographed to homogeneity.

D. *N*- α -Acylation of Histidine by Active Esters. Boc-His(DNP)-OH¹³ (15.4 mmol) was dissolved in 50 mL of 1,2-dimethoxyethane, and 310 mL of 5.5 M HCl in 1,2-dimethoxyethane was added at 0 °C. The reaction mixture was stirred at room temperature for 5 h and then evaporated. The crude product was dissolved in 300 mL of THF/ethanol (1:2), and 14.1 mmol of

N-terminal hydroxysuccinimide ester (prepared from the corresponding acid via the standard procedure²¹) was added. Saturated aqueous NaHCO_3 (240 mL) was added, and the mixture was stirred at room temperature for 24 h. The organic solvents were evaporated, acidified to pH 2 with 5% aqueous NaHSO_4 , and extracted with 3 \times 200 mL of ethyl acetate. The organic layer was dried and evaporated. The resulting residue was dissolved using 80 mL of acetone/ethyl acetate (1:1), and the desired product was precipitated by addition of 500 mL of diethyl ether to yield a yellow amorphous solid which was used without further purification.

E. Preparation of Protected Mannofuranosyl *N*-Glycosides. A suspension of 300 g (1.67 mol) of D-(+)-mannose and a catalytic amount (1 g) of 4-toluenesulfonic acid in 1.2 L of 2,2-dimethoxypropane was stirred at 40 °C for 4 h. The mixture turned into a clear solution, and 1.67 mol of amine was added. The mixture was refluxed for 24 h, 500 mL of 2,2-dimethoxypropane was added, and the mixture was refluxed for another 24 h. The reaction mixture was evaporated and chromatographed to homogeneity.

In Vitro Enzyme Inhibition Assays. Purified human kidney renin was assayed using the tetradecapeptide Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Asn at pH 7.5. Plasma assays were performed as described previously²² except for the system was buffered at pH 7.4. The inhibition of pepsin and cathepsin D was measured as described previously.²³

Rhesus Monkey. The experiments were carried out in six rhesus monkeys of either sex, weighing between 5.0 and 10.5 kg. Prior to onset of experiments sodium depletion was achieved by oral administration of 10 mg/kg per day furosemide-Na for 6 consecutive days. At day 7, 10 mg/kg furosemide was given iv about 30 min before the experiment was started. Anesthesia was introduced with 20 mg/kg ketamine hydrochloride im and continued with 40 mg/kg pentobarbitone-Na slowly iv. A small side branch of the femoral or radial artery was surgically exposed and cannulated for blood pressure measurements using a pressure transducer (P23 ID). Heart rate was determined from a conventional ECG lead by a biotachymeter. Blood samples were withdrawn via a braunule placed into the saphenous vein. A gastric fibroscope (Olympus XP 10) was introduced into the duodenum under visual control and 33 was administered through the fibroscope's service channel. 33 was dissolved in 0.1 M citric acid at a concentration of 2 mg/mL. The complete volume of administered liquid including rinsing amounted to 5 mL. Blood samples were withdrawn before and at 15, 30, 45, 60, 75, 90, and 120 min after the intraduodenal administration. After all data and blood samples had been obtained, the gastric fibroscope was withdrawn. The artery was closed by compression for some minutes and the animal was allowed to recover.

Transport Studies Using Brush Border Membrane Vesicles. The preparation of brush border membrane vesicles from

- (21) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. The Use of Esters of *N*-Hydroxysuccinimide in Peptide Synthesis. *J. Am. Chem. Soc.* 1964, 86, 1839–1842.
- (22) Bolis, G.; Fung, A. K. L.; Greer, J.; Kleinert, H. D.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. H. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Incorporating Transition-State, Nonpeptidic Replacements at the Scissile Bond. *J. Med. Chem.* 1987, 30, 1729–1737.
- (23) Mycek, M. Cathepsins. In *Methods of Enzymology*; Perlmann, G., Lorand, L., Eds.; Academic: New York, 1970; Vol. XIX, p 286.
- (24) Abbreviations for the amino acids follow IUPAC-IUB Commission on Biochemical Nomenclature guidelines: *Eur. J. Biochem.* 1984, 138, 9–37. Additional abbreviations used herein include: 2,2-dimethoxypropane, DMP; 4-toluenesulfonic acid, *p*-TosOH; 2(*S*)-[[[*N*-methyl-*N*-(2-[*N*-(morpholinocarbonyl)-*N*-methylamino]ethyl)amino]carbonyl]oxy]-3-phenylpropionic acid, MMP; di-*tert*-butyl dicarbonate, Boc_2O ; bis(1-benzotriazolyl) carbonate, [(HOBT) $_2\text{CO}$]; trifluoroacetic acid, TFA; *cis*-4-aminocyclohexanecarboxylic acid, ACC; *n*-propylphosphonic anhydride, PPA; 2-benzyl-3-(*tert*-butylsulfonyl)propionic acid, BBP; isovaleroyl, Iva; diisopropyl ether, DIP; *tert*-butylmethyl ether, MTB; ethyl acetate, EE; diphenyl phosphorazidate, DPPA; *O*-(benzotriazol-1-yl)-*N*,*N*,*N*'-tetramethyluronium hexafluorophosphate, HBTU.

rabbit small intestine as well as transport studies and photoaffinity labeling experiments were performed as described previously.^{16,17}

Stability Study. 33 (2.5 mg) was dissolved in 0.1 mL of methanol, and 29 mg of NaCl and the amount of phosphate buffer pH 7 were added to yield 5 mL of test solution; 0.5 mL was removed, added to 0.5 mL of acetonitrile containing 0.1% trifluoroacetic acid, and taken as the reference ($T = 0$). The remaining test solution was then incubated with 4.5 mg of α -chymotrypsin at 37 °C. Aliquots of 0.2 mL each were taken at intervals and quenched by addition of 0.2 mL of acetonitrile (0.1%

TFA). Analyses of the incubation were carried out by reversed-phase HPLC on nucleosil C-18 (7 μ m) eluted using 60% MeOH/40% H₂O/0.1% ammonium acetate. Peak detection was by UV absorbance at 254 nm with quantification using a Gilson Data Master. $T_{1/2}$ turned out to be about 4 h.

Acknowledgment. The synthetic assistance of Martina Mollath and Peter Voss and the technical support of W. Jung and M. Platil with id monkey experiments is gratefully acknowledged.

Base-Catalyzed Isomerization of Retinoic Acid. Synthesis and Differentiation-Inducing Activities of 14-Alkylated *all-trans*-, 13-*cis*-, and 20,14-*retro*-Retinoic Acids

Hideo Tanaka,[†] Hiroyuki Kagechika,[†] Emiko Kawachi,[†] Hiroshi Fukasawa,[†] Yuichi Hashimoto,[‡] and Koichi Shudo^{*†}

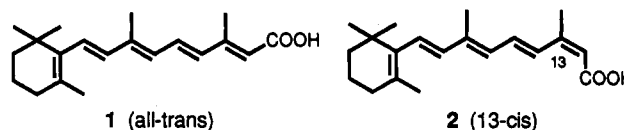
Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan, and Institute of Applied Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan. Received June 11, 1991

Retinoic acid (1) is isomerized regioselectively by excess amounts of lithium diisopropylamide (LDA) to give 20,14-*retro*-retinoic acid (3). Alkylation of the intermediate dianion of retinoic acid gave 14-alkylated derivatives of 3. By isomerization of the alkylated retro isomers under basic conditions, several 14-alkyl-*all-trans*- and -13-*cis*-retinoic acids were synthesized. The retinoid activities of these derivatives were examined, based on the ability to induce differentiation of human promyelocytic leukemia cell line HL-60. 20,14-*retro*-Retinoic acid (3) is 1/50 as active as retinoic acid (1). Although 14-methyl-20,14-*retro*-retinoic acid (4) is as active as 3, the introduction of a 14-methyl group into *all-trans*- and 13-*cis*-retinoic acid resulted in decreased activity. Introduction of bulkier alkyl groups at the C-14 position caused the disappearance of the activity.

Retinoic acid (*all-trans*, 1) plays fundamental roles in cell differentiation and proliferation^{1,2} and is a morphogen in chicks³ and amphibia.⁴ Elucidating its mechanism of action is currently considered to be one of the most important problems in biology. Recently, it has been established that retinoic acid (1) binds to its specific nuclear receptor(s) in order to regulate specific gene expressions,⁵ being similar in that respect to the steroid hormones, thyroid hormone, and vitamin D₃. Now, retinoic acid (1) is regarded as an internal "hormone" rather than a vitamin.

In contrast to the recognition of the significant biological action of retinoic acid (1), its chemical behavior has not been systematically studied. Retinoic acid (1) has a unique structure, consisting of a conjugated pentaenoic acid system, and is expected to undergo various types of reactions. Among them, isomerization of retinoic acid (1) is one of the more interesting reactions from the viewpoint of pure chemistry. It is also useful in connection with the synthesis of biologically related derivatives, which might be useful clinically.⁶ Retinoic acid (1) is isomerized photochemically and thermally to give a number of products including 13-*cis*-retinoic acid (2, Chart I),⁷ but acid- and base-catalyzed isomerizations have scarcely been studied. We previously reported that retinoic acid (1) isomerizes under acidic conditions to give complex mixtures consisting of annulated isomers.⁸ In this paper, we report the regioselective isomerization of retinoic acid catalyzed by LDA, which forms 20,14-*retro*-retinoic acid (3) in good yield. Furthermore, using the anionic intermediate of this reaction, several derivatives with an alkyl group at the C-14 position of *all-trans*-, 13-*cis*-, and 20,14-*retro*-retinoic acid were synthesized. The retinoid activity of these compounds was examined, on the basis of ability to induce differentiation of human promyelocytic

Chart I



leukemia cell line HL-60 to mature granulocytes.^{9,10} This ability of retinoids can be measured very sensitively and

- (1) Sporn, M. B.; Roberts, A. B.; Goodman, D. S., Eds. *The Retinoids*; Academic Press, Inc.: Orlando, FL, 1984.
- (2) Dawson, M. I.; Okamura, W. H., Eds. *Chemistry and Biology of Synthetic Retinoids*; CRC Press: Boca Raton, FL, 1990.
- (3) Thaller, C.; Eichele, G. Identification and Spatial Distribution of Retinoids in the Developing Chick Limb Bud. *Nature* 1987, 327, 625-628.
- (4) Giguere, V.; Ong, E. S.; Evans, R. M.; Tabin, C. J. Spatial and Temporal Expression of the Retinoic Acid Receptor in the Regenerating Amphibian Limb. *Nature* 1989, 337, 566-569.
- (5) (a) Petkovich, M.; Brand, N. J.; Kurst, A.; Chambon, P. A Human Retinoic Acid Receptor which Belongs to the Family of Nuclear Receptors. *Nature* 1987, 330, 444-450. (b) Giguere, V.; Ong, E. S.; Segui, P.; Evans, R. M. Identification of a Receptor for the Morphogen Retinoic Acid. *Nature* 1987, 330, 624-629. (c) Brand, N.; Petkovich, M.; Kurst, A.; Chambon, P.; de The, H.; Marchio, A.; Tiollais, P.; Dejean, A. Identification of a Second Human Retinoic Acid Receptor. *Nature* 1988, 332, 850-853. (d) Hashimoto, Y.; Kagechika, H.; Kawachi, E.; Shudo, K. Specific Uptake of Retinoids into Human Promyelocytic Leukemia Cells HL-60 by Retinoid-specific Binding Protein: Possibly the True Retinoid Receptor. *Jpn. J. Cancer. Res.* 1988, 79, 473-483. (e) Hashimoto, Y.; Petkovich, M.; Gaub, M. P.; Kagechika, H.; Shudo, K.; Chambon, P. The Retinoic Acid Receptors α and β are Expressed in the Human Promyelocytic Leukemia Cell Line HL-60. *Mol. Endocrinol.* 1989, 3, 1046-1052. (f) Krust, A.; Kastner, P.; Petkovich, M.; Zelent, A.; Chambon, P. A Third Human Retinoic Acid Receptor, hRAR- γ . *Proc. Natl. Acad. Sci. U.S.A.* 1989, 86, 5310-5314. (g) Hashimoto, Y.; Kagechika, H.; Shudo, K. Expression of Retinoic Acid Receptor Genes and the Ligand-Binding Selectivity of Retinoic Acid Receptors (RAR's). *Biochem. Biophys. Res. Commun.* 1990, 166, 1300-1307.

[†] Faculty of Pharmaceutical Sciences.

[‡] Institute of Applied Microbiology.