Renin Inhibitory Pentols Showing Improved Enteral Bioavailability†

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Incorporation of a C-terminal pentahydroxy functionality led to potent, low molecular weight hydrophilic renin inhibitors lacking the P_1' 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 investigations[3] 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 diacetonide **2**[4] 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 $TiCl_4$ and benzylamine[5] 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 hydrogenation[6] 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 addition[7] 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*-(morpholinocarbonyl)-N-methylamino]ethyl]amino]carbonyl]oxy]-3-

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

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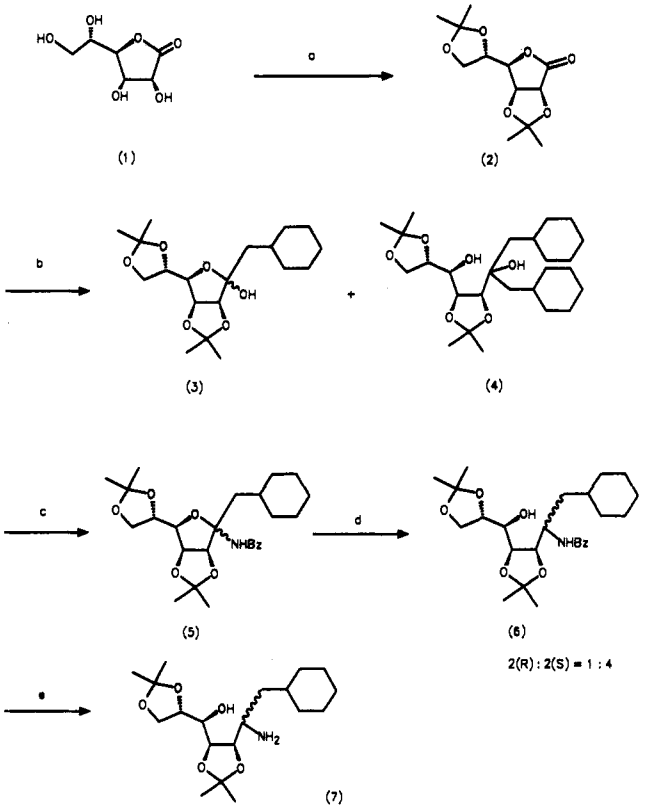
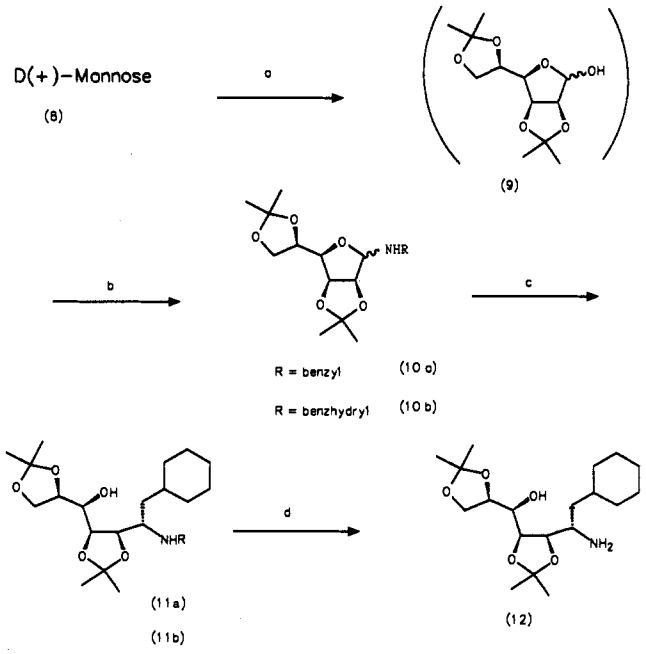
Scheme I[a]



[a] (a) DMP, *p*-TosOH; (b) (cyclohexylmethyl)magnesium bromide; (c) $TiCl_4$, benzylamine; (d) $LiAlH_4$; (e) $HCO_2^-NH_4^+$, Pd/C.

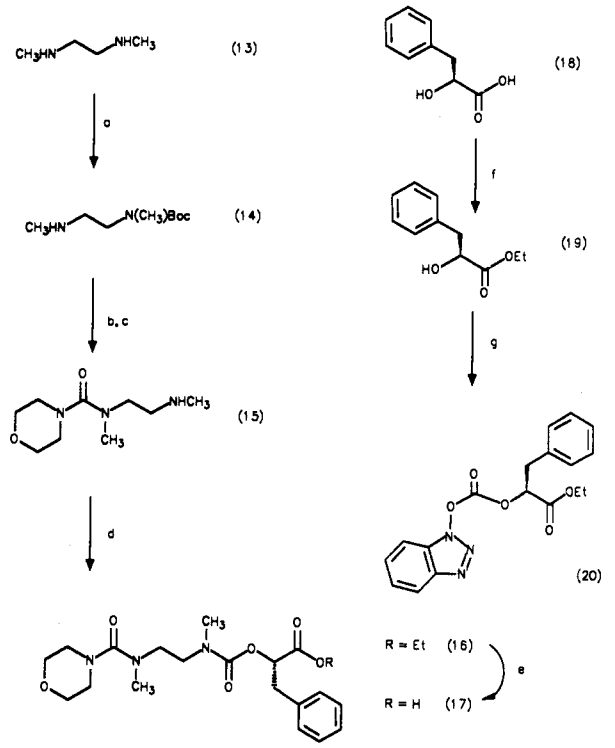
Scheme II[a]



[a] (a) DMP, *p*-TosOH; (b) R-NH_2; (c) (cyclohexylmethyl)lithium; (d) $HCO_2^-NH_4^+$, Pd/C.

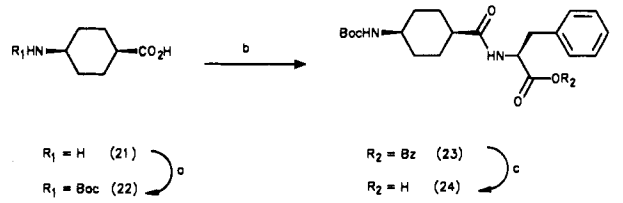
phenylpropionic acid (MMP) (**17**) is outlined in Scheme III. *N,N'*-Dimethylethylenediamine (**13**) was monoprotected to **14** using di-*tert*-butyl dicarbonate (Boc_2O). The unsymmetrical urea **15** was synthesized by sequential treatment of **14** with bis(1-benzotriazolyl) carbonate [$(HOBT)_2CO$] 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 Boc_2O followed by *n*-propylphosphonic anhydride (PPA) coupling[10] to phenylalanine benzyl ester and subsequent hydrogenation gave N-terminal **24**.

Scheme III[a]



[a] (a) Boc_2O; (b) 1-morpholino-4-(carbonyloxy)benzotriazole; (c) TFA; (d) **20**; (e) NaOH; (f) HCl/EtOH; (g) $(HOBT)_2CO$.

Scheme IV[a]



[a] (a) Boc_2O; (b) H-Phe-OBz, PPA; (c) H_2, Pd/C.

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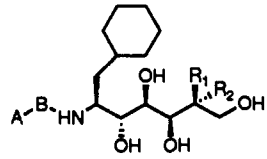
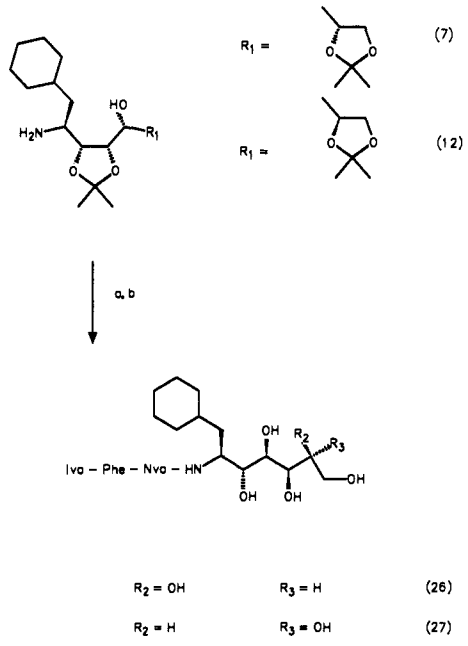
Table I. Renin Inhibitory Potency of Pentols



compd	A	B	R_1	R_2	IC_{50} [nM] kidney	IC_{50} [nM] 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

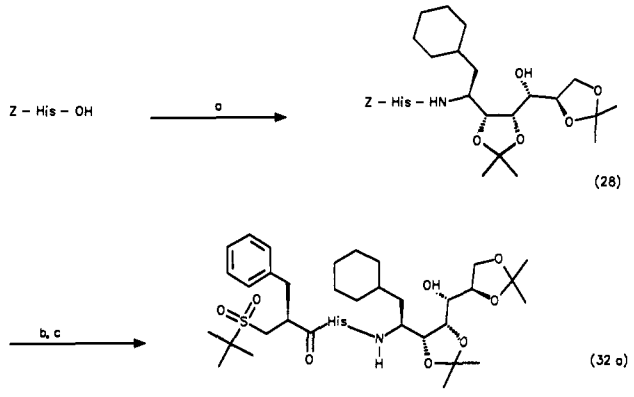
[a] See Scheme VII.

Scheme V[a]



[a] (a) Iva-Phe-Nva-OH (**25**), pivaloyl chloride, base; (b) *p*-TosOH, methanole.

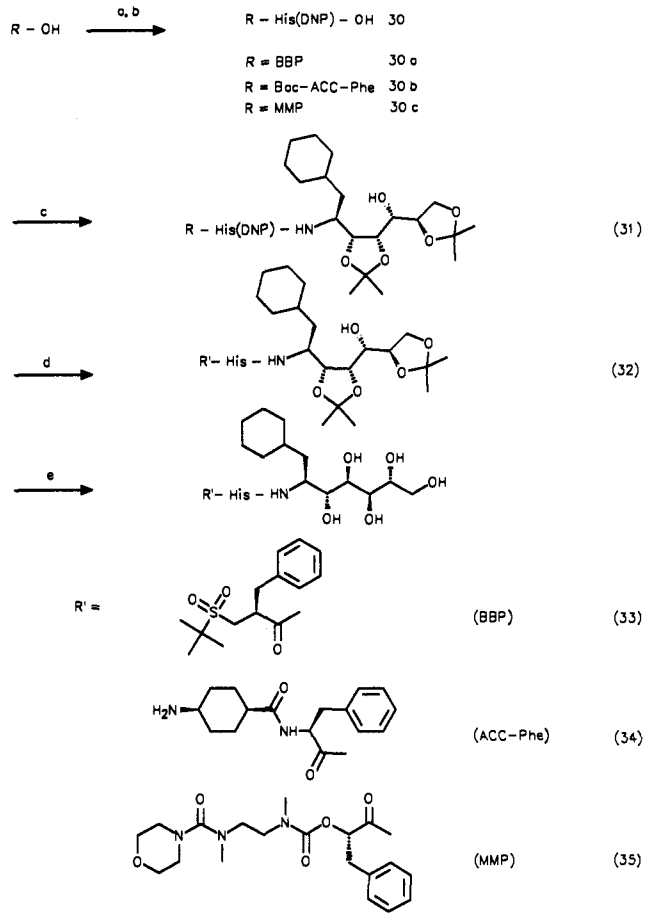
Scheme VI[a]



[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) containing inhibitors were synthesized by stepwise build-up via an azide coupling[12] 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 group[13] on the imidazole moiety, was coupled

Scheme VII[a]



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

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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	$>10^{-4}$		
rat plasma renin	8.0×10^{-6}	rhesus monkey plasma renin	1.1×10^{-8}
dog plasma renin	1.1×10^{-7}	human plasma renin	4.5×10^{-9}

with *manno*-pentol 12 using pivaloyl chloride[14] 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 shown[8] 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

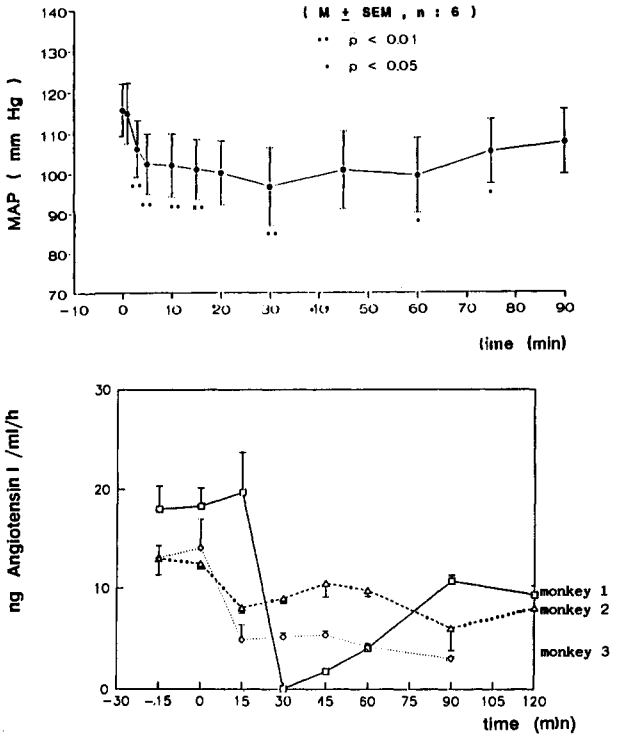

Figure 1. Effects of 2 mg/kg **33** id on MAP and plasma renin activity in sodium-depleted anesthetized rhesus monkeys.

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 cephalexin was used as a prototype substrate for the oligopeptide/β-lactam antibiotic transporter. The H^+-dependent uptake of 2 mM cephalexin into rabbit small intestine brush border membrane vesicles was inhibited by compound **33** in a concentration-dependent manner with an IC_{50} of 5–8 mM. On the other hand, the uptake of **33** into brush border membrane vesicles was inhibited by increasing concentrations of cephalexin, 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 [^{3}H]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

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concentration-dependent manner (IC_{50} = 3 mM at a glucose concentration of 20 μM). 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 fiberscope 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 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, 0.04–0.063 mm (E. Merck), eluting with 5–20 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 124–127 °C [TLC R_f 0.33]; $[\alpha]_D$ 12.4° (c 1.0, methanol), besides 4.3 g (11%) [TLC R_f 0.44] of **(3*R*,4*R*,5*R*,6*S*)-1-cyclohexyl-2-(cyclohexylmethyl)-2,3,4,5,6,7-hexahydroxyheptane 3,4:6,7-diacetonide (4)**. **3**: ^1H NMR ($CDCl_3$) δ 0.95–1.95 (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 Hz), 4.06 (dd, 1 H, J^1 = 4.0 Hz, J^2 = 8.0 Hz), 4.22 (dd, 1 H, J^1 = 7.0 Hz, J^2 = 6.0 Hz), 4.30 (m, 1 H), 4.47 (d, 1 H, J = 6 Hz), 4.71 (dd, 1 H, J^1 = 4 Hz, J^2 = 7 Hz). **4**: ^1H NMR ($CDCl_3$) δ 0.90–1.10 (m, 4 H), 1.15–1.30 (m, 6 H), 1.32 (s, 3 H), 1.39 (s, 3 H), 1.45 (s, 3 H), 1.58 (s, 3 H), 1.40–1.85 (m, 16 H), 1.97 (d, b, 1 H, J = 13.2 Hz), 3.72 (dd, 1 H, J^1 = J^2 = 8.0 Hz), 3.88–3.97 (m, 3 H), 4.02 (d, 1 H, J = 6.0 Hz), 4.11 (dd, 1 H, J^1 = 6.0 Hz, J^2 = 8.0 Hz), 4.28–4.38 (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, $[\alpha]_D$ 4.0° (c = 1.0, methanol). Because of its instability, it was always used immediately after preparation.

(2*RS*,3*R*,4*R*,5*R*,6*S*)-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 2*S* and 2*R* diastereoisomers. The major isomer was assumed to be 2*S* because of biological activity.

(2*RS*,3*R*,4*R*,5*R*,6*S*)-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*-Isovaleroyl-Phe-Nva [(2*S*,3*R*,4*R*,5*R*,6*S*)-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*-isovaleroyl-Phe-Nva [(2*S*,3*R*,4*R*,5*R*,6*S*)-1-cyclohexyl-3,4:6,7-di-*O*-isopropylidene-3,4,5,6,7-pentahydroxyhept-2-yl]amide as a colorless foam [TLC R_f 0.07], besides 90 mg (8%) of the 2*R* product [TLC R_f 0.15]: ^1H NMR ($CDCl_3$) δ 0.78–1.05 (m, 11 H), 1.10–1.50 (m, 8 H), 1.60–1.90 (m, 6 H), 1.37 (s, 3 H), 1.42 (s, 3 H), 1.44 (s, 3 H), 1.60 (s, 3 H), 1.95–2.07 (m, 3 H), 2.82 (d, 1 H, J = 10.4 Hz), 3.03 (dd, 1 H, J_1 = 8.4 Hz, J_2 = 14.0 Hz), 3.18 (dd, 1 H, J_1 = 6.0 Hz, J_2 = 14.0 Hz), 3.69–3.78 (m, 1 H), 3.95 (dd, 1 H, J_1 = J_2 = 8.0 Hz), 4.08 (dd, 1 H, J_1 = J_2 = 8.0 Hz), 4.12–4.30 (m, 5 H), 4.66–4.76 (m, 1 H), 5.86 (d, 1 H, J = 8.0 Hz), 6.30 (d, 1 H, J = 9.2 Hz), 6.78 (d, 1 H, J = 7.6 Hz), 7.15–7.34 (m, 5 H).

A 140-mg (0.20-mmol) portion of the thus obtained diacetonide was deprotected using general procedure **B**. Chromatography with CH_2Cl_2/methanol (8:1) [TLC R_f 0.22] yielded 46 mg (37%) of the desired product as amorphous solid: ^1H NMR (DMSO-d_6) δ 0.67 (d, 3 H, J = 6.8 Hz), 0.73 (d, 3 H, J = 6.5 Hz), 0.88 (t, 3 H, J = 7.2 Hz), 1.10–1.35 (m, 8 H), 1.45–1.90 (m, 8 H), 2.10 (d, 2 H, J = 8.5 Hz), 2.95–3.60 (m, 6 H), 3.93 (d, 1 H, J = 7.2 Hz), 4.05–4.15 (m, 2 H), 4.20–4.32 (m, 1 H), 4.39–4.45 (m, 1 H), 4.47 (d, 1 H, J = 7.3 Hz), 4.50–4.65 (m, 3 H), 4.85 (d, 1 H, J = 7.3 Hz), 7.15–7.29 (m, 5 H), 7.40 (d, 1 H, J = 9.2 Hz), 7.99 (d, 1 H, J = 8.7 Hz), 8.05 (d, 1 H, J = 8.0 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. ^1H 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 Hz, J^2 = 3.2 Hz), 3.76–4.00 (m, 2 H), 4.06–4.16 (m, 3 H), 4.38–4.45 (m, 1 H), 4.56 (dd, 1 H, J^1 = 6.4 Hz, J^2 = 3.2 Hz), 4.68 (dd, 1 H, J^1 = 6.4 Hz, J^2 = 3.2 Hz), 7.22–7.42 (m, 5 H).

(2*S*,3*R*,4*R*,5*R*,6*R*)-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 (Θ = 3.2 mm, high Na), and 40.2 g (0.23 mol) of cyclohexylmethyl bromide were dissolved in 1.1 L of anhydrous tetrahydropyran 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 2*R* 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).

(2*S*,3*R*,4*R*,5*R*,6*R*)-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 2*R* product was detectable.)

(2*S*,3*R*,4*R*,5*R*,6*R*)-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 [(2*S*,3*R*,4*R*,5*R*,6*R*)-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 [(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 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 (DMSO-d_6/D_2O) 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)-OH[16] (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 [(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 (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 [(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 (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 [(2*S*,3*R*,4*R*,5*R*,6*R*)-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 [(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 (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 [(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 (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: ^{1}H 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; ^{1}H 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]: ^{1}H 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: ^{1}H 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)-OH[16] (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: ^{1}H 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 diacetonide 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: ^{1}H 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). Diacetonide 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: ^{1}H 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-aminocyclohexane-carboxylic 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; ^{1}H 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; ^{1}H 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; ^{1}H 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-pentahydroxyhept-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: ^{1}H 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-pentahydroxyhept-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 × 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: ^{1}H NMR (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)-OH[13] (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 procedure[21]) 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 × 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 previously[22] 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 fiberscope (Olympus XP 10) was introduced into the duodenum under visual control and **33** was administered through the fibercope'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 fiberscope 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

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(24) Abbreviations for the amino acids follow IUPAC-IUB Commision 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)$_2$CO]; trifluoracetic 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*′,*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 μm) eluted using 60% MeOH/40% H_2O/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.

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

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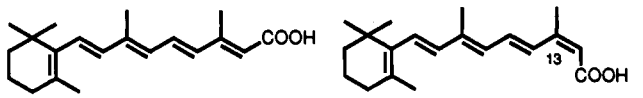
Retinoic acid (1) is isomerized regioselectively by excess amounts of lithium diisopropylamide (LDA) to give 20,14-*retro*-retinoic acid (3). Alkylation of the intermediate dianion of retinoic acid gave 14-alkylated derivatives of 3. By isomerization of the alkylated retro isomers under basic conditions, several 14-alkyl-*all-trans*- and -13-*cis*-retinoic acids were synthesized. The retinoidal 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 chicks[3] 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 D_3. 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 an 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 retinoidal activity of these compounds was examined, on the basis of ability to induce differentiation of human promyelocytic leukemia cell line HL-60 to mature granulocytes.[9,10] This ability of retinoids can be measured very sensitively and

Chart I



1 (all-trans) 2 (13-cis)

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