acid composition of $Asp_{0.96}Pro_{0.99}Val_{2.34}Tyr_{0.25}Phe_{1.14}His_{0.80}Arg_{1.00}$. Part of the sample (0.35 g) was then dissolved in 2 ml of TFA. Anisole (0.35 ml) was added and the mixture treated with 8 ml of anhydrous HF for 60 min at 0°. Removal of the excess acids left an oily residue which was taken up in 60 ml of water, washed several times with ether, and lyophilized to give 0.17 g of crude peptide. It was then purified by countercurrent distribution in a solvent system made up from *n*-BuOH–HOAc–H_2O (4:1:5) for 200 transfers (K = 0.27) followed by gel filtration on a Sephadex G-10 column (2.5 × 85 cm) using 0.2 M acetic acid as eluent. The material in the major peak was collected and lyophilized to give 56 mg of pure product. It was shown to be homogeneous on thin layer chromatography and paper electrophoresis. On acid hydrolysis, the compound gave the correct amino acid analysis: $Asp_{1.06}Pro_{1.03}Val_{1.93}Tyr_{1.02}Phe_{1.01}His_{0.93}Arg_{1.00}$.

Anal. Calcd for $C_{51}H_{83}N_{13}O_{19}$ (1182.3): C, 51.81; H, 7.08; N, 15.40. Found: C, 51.84; H, 7.06; N, 15.47.

Bpoc-Leu-Met-OCH_3 (XXIV). Bpoc-L-Leu (6.9 g, 18.6 mmol) was allowed to react with 3.73 g of Met-OCH_3·HCl (18.7 mmol) and 3.9 g of DCC in 80 ml of CH_2Cl_2 containing 2.6 ml of triethylamine at 0° for 2 hr. The insoluble by-product formed was filtered off and the filtrate washed a few times with water. The solution was dried over Na_2SO_4 and then concentrated to an oil. It was dissolved in a small volume of CH_2Cl_2 and treated with petroleum ether. Upon cooling, the product started to crystallized slowly: yield, 7.5 g (78%); mp 80–82°; $[\alpha]^{25}D$ −38.09° (c 1, MeOH).

Anal. Calcd for $C_{26}H_{38}N_2O_5S$ (514.7): C, 65.34; H, 7.44; N, 5.44. Found: C, 65.73; H, 7.83; N, 5.38.

Bpoc-Leu-Met-NH_2. Ammonolysis of the above compound (XXIV, 3 g) in 80 ml of methanol that had been saturated with dry ammonia resulted in the formation of the corresponding peptide amide (1.96 g, 67%): mp 99–102°; $[\alpha]^{25}D$ −37.46° (c 1, MeOH).

Anal. Calcd for $C_{17}H_{37}N_3O_4S$ (499.7): C, 64.90; H, 7.46; N, 8.41. Found: C, 64.90; H, 7.78; N, 8.27.

Z-Lys(Z)-Phe-Phe-Gly (XX). Bpoc-Gly-resin XIX (7.0 g, 2.52 mmol) was placed in the peptide synthesis flask and the solid phase synthesis[15] carried out with 150-ml portions of solvents using a 2.4-fold excess of amino acid derivative and DCC in each cycle. As outlined in Scheme V, Bpoc-L-Phe (2.42 g), Bpoc-L-Phe (2.42 g), and Z-L-Lys(Z) (2.43 g) were sequentially coupled to the resin to give 7.5 g of the protected tetrapeptide resin. The peptide was then released from the resin by stirring in 150 ml of 50% TFA for 30 min. After removing the resin particles and the solvents, the oily residue left was treated with 50 ml of ethyl acetate. The solid obtained was dissolved in THF and crystallized by addition of water: yield, 1.02 g (60%); mp 220–222°; $[\alpha]^{25}D$ −25.55° (c 1, DMF); nmr spectrum consistent with the structure.

Anal. Calcd for $C_{42}H_{47}N_5O_9$ (765.9): C, 65.87; H, 6.19; N, 9.14. Found: C, 65.81; H, 6.19; N, 9.14.

Z-Lys(Z)-Phe-Phe-Gly-Leu-Met-OCH_3 (XXI). Bpoc-Leu-Met-OCH_3 (XXIV) (0.52 g, 1.0 mmol) was dissolved in a mixture of 1 ml of 2.4 N HCl in ethyl acetate and 47.5 ml of CH_2Cl_2. After 10-min standing, the solvents were removed at 25° under reduced pressure and the oily residue of the dipeptide hydrochloride was taken up in 20 ml of DMF–CH_2Cl_2 mixture. The solution was cooled to 0° while 0.77 g of Z-Lys(Z)-Phe-Phe-Gly (1 mmol) was added followed immediately by 0.3 ml of N-methylmorpholine and 0.23 g of DCC. The mixture was stirred at 0° overnight. The insoluble material formed was filtered off and the filtrate washed a few times with water, dried over Na_2SO_4, and then evaporated to an oil. It was dissolved in DMF–CH_2Cl_2 mixture and precipitated with ether. The product was crystallized from THF by slow addition of water: yield, 0.85 g (83%), mp 180–184°.

Anal. Calcd for $C_{54}H_{69}N_7O_{11}S$ (1024.3): C, 63.32; H, 6.79; N, 9.57. Found: C, 63.87; H, 6.74; N, 9.54.

Z-Lys(Z)-Phe-Phe-Gly-Leu-Met-NH_2 (XXII). The above compound XXI (0.75 g, 0.73 mmol) was suspended in 100 ml of dry methanol and bubbled with dry ammonia gas for 2 hr at 0°. The compound became soluble in the solution but started to crystallize out slowly during overnight standing at room temperature. The product was collected and washed with ether to give 0.58 g of the desired compound: mp 238–242°; $[\alpha]^{25}D$ −39.28° (c 1, DMF).

Anal. Calcd for $C_{53}H_{68}N_8O_{10}S$ (1009.3): C, 63.08; H, 6.70; N, 11.10; S, 3.18. Found: C, 62.79; H, 6.70; N, 11.25; S, 2.90.

Lys-Phe-Phe-Gly-Leu-Met-NH_2 (XXIII). Compound XXII (0.15 g) was dissolved in 10 ml of TFA containing 0.5 ml of mercaptoethanol as well as 1 ml of anisole. The mixture was warmed at 80° for 3 hr during which time some white insoluble material came out of the solution. It was fitered off and the filtrate was treated with a large volume of ether to precipitate the product. The crude peptide was then purified by countercurrent distribution in a solvent system of *n*-BuOH–HOAc–pyridine–H_2O (8:2:2:9) for 300 transfers (K = 2.8) followed by gel filtration on a Sephadex G-10 column (2.5 × 85 cm) using 0.2 M acetic acid as eluent. The material in the main fraction was collected and lyophilized to give 33 mg of pure product. It gave correct amino acid analyses upon acid hydrolysis: $Gly_{1.00}Met_{0.93}Leu_{1.05}Phe_{2.11}Lys_{0.92}$. The product was shown to be homogeneous on thin layer chromatography and paper electrophoresis.

Anal. Calcd for $C_{37}H_{56}N_8O_6S \cdot 2CH_3COOH$ (861.1): C, 57.19; H, 7.49; N, 13.01. Found: C, 57.38; H, 7.61; N, 13.00.

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Communications to the Editor

The Reverse Anomeric Effect and the Synthesis of α-Glycosides

Sir:

One of the classical problems of carbohydrate chemistry has been the preparation of α-glycosides—especially those of cis-1,2 configuration. The problem has been difficult since the usual substituents used as leaving groups on C-1 are electronegative and have a preferred axial or α configuration because dipolar interactions predominate over the usual steric factors. Therefore, when nonparticipating groups are present on C-2 and replacement by alkoxyl occurs with inversion, β-glycosides are usually the preferred product. When participating groups are present on C-2, the configuration of the resulting glycoside is predominantly determined by participation of the C-2 substituent and the product is largely trans 1,2. The picture is further complicated by the partial carbonium ion character of the intermediate, partial inversion of the reagent by negative ion before glycoside formation, steric hindrance, the probable participation of groups on other sites, and the possibility in some instances of ortho ester formation.

Variable yields of α-linked glycosides have been prepared in select cases by controlling one or all of the above-mentioned factors that influence the stereoselectivity of the reaction employed. One of the more promising recent approaches to α-glycoside syntheses was originated by Ishikawa and Fletcher[1] and is being extended by others.[2,3]

In these and other examples, α-glycosides have been prepared by controlling the possible participation of groups on sites other than C-2,[2-4] the C-1 configura-

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Table I. Results of Methanolysis of the Reactive Intermediates from Salt-Forming Reactions

Glucopyranosyl	Reaction	Nucleophile	C_1 salt intermediate	Solvent	Methanolysis temp, °C	% anomeric ratio of methyl glucopyranosides[c]
TBG[a]	I	$N(C_2H_5)_3$	Ammonium	$N(C_2H_5)_3$	25	100 α
TBG	I	$N(C_2H_5)_3$	Ammonium	$O(C_2H_5)_2$	25	100 α
TBG	II	$S(CH_3)_2$	Sulfonium	$S(CH_3)_2$	25	86 α/14 β
TBG	II	$S(CH_3)_2$	Sulfonium	$O(C_2H_5)_2$	25	86 α/14 β
TBG	III	PPh_3	Phosphonium	$O(C_2H_5)_2$	40	100 α
BTBG[b]	IV	$N(C_2H_5)_3$	Ammonium	$N(C_2H_5)_3$	25	100 α
BTBG	IV	$N(C_2H_5)_3$	Ammonium	$O(C_2H_5)_2$	25	100 α

[a] 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl bromide. [b] 2,3,4-Tri-*O*-benzyl-6-*O*-*p*-methoxybenzoyl-α-D-glucopyranosyl bromide. [c] Anomeric ratio determined as outlined in ref 3.

tion of the starting glycosyl halide,[5-7] the nature of the C-1 leaving group,[8-10] the conditions of the Koenigs–Knorr reaction,[7,8,11-18] and the use of mercuric salts.[2,19-22] These methods, however, rarely give very high selectivities and do not appear to suggest a general approach.

Since a stereoselective reaction course is most readily obtained in reactions occurring with inversion, it appeared advisable to us to search for a C-1 leaving group, the thermodynamically preferred configuration of which would be equatorial. For this, the effect of dipolar interaction must support the usual steric factors and the substituent must, therefore, be electropositive.[23] The presence of an electropositive leaving group has the further advantage of making C-1 susceptible to nucleophilic attack.

A typical application of this approach to a glycosyl derivative with an electronegative C-1 substituent and nonparticipating blocking group at C-2 would then involve a double inversion. The first step would be reaction with a neutral nucleophile that could not readily lose proton or another positive fragment. The resultant product should be a positively charged sugar moiety with the original leaving group as gegenion. The next step would be reaction with a second nucleophile (preferably one without strong proton abstracting ability) as, for example, an alcoholic function.

We wish to report four successful examples of this reaction scheme. 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl bromide[1] (TBGB) (0.34121 g, 5.66×10^{-4} mol) was allowed to react with excess triethylamine (3.6275 g, 3.59×10^{-2} mol) (reaction I) or dimethyl sulfide (4.23 g, 6.82×10^{-2} mol) (reaction II), or an anhydrous diethyl ether solution (5 ml) of triphenylphosphine (0.15585 g, 5.93×10^{-4} mol) (reaction III) in evacuated sealed tubes at or near ambient temperature. Similarly, 2,3,4-tri-*O*-benzyl-6-*O*-*p*-methoxybenzoyl-α-D-glucopyranosyl bromide[3] (BTBGB) (0.18918 g, 2.924×10^{-4} mol) was allowed to react with excess triethylamine (3.6275 g, 3.59×10^{-2} mol) (reaction IV). In all examples a cloudy solution was observed within 1 hr of mixing with precipitation occurring after being set aside in the dark for 12 hr. Removal of the respective solvents yielded a syrupy product for reactions I, II, and IV, while a white amorphous solid was obtained from reaction III. The isolated products are insoluble in anhydrous diethyl ether (unlike the initial bromide) but soluble in methylene chloride and chloroform. All of these intermediates are extremely sensitive to moisture and light. Attempts to induce crystallization and obtain analytically pure samples for analysis failed. However, the more stable products isolated from reactions I and III did lend themselves to partial characterization.

The syrup isolated from reaction I was washed with anhydrous diethyl ether and anhydrous petroleum ether (bp 20–60°) to remove unreacted amine. The resulting syrup was dried under high vacuum to a constant weight and had $[\alpha]^{25}D$ −2.75° (*c* 1.164, chloroform). Its 60-MHz nmr spectrum ($CDCl_3$) included a broad multiplet at δ 5.52 ppm worth one proton. The ratio of aromatic resonances to nonaromatic proton resonances was consistent with a 1:1 adduct of triethylamine to the starting bromide. Similarly, the solid isolated from reaction III was washed with anhydrous diethyl ether and anhydrous petroleum ether (bp 30–60°) to remove the unreacted triphenylphosphine and dried under high vacuum to a constant weight. The resulting solid had $[\alpha]^{25}D$ +18.53° (*c* 1.36, chloroform) and melted at 180–182° with decomposition. Its 60-MHz nmr spectrum ($CDCl_3$) included a doublet ($J_{H,P}$ = 14 Hz) centered at δ 5.28 ppm worth one proton. The ratio of aromatic proton resonances to glucoside proton resonances was in agreement with a 1:1 adduct of the starting bromide with triphenylphosphine.

Reaction of any of these intermediates (the anomeric configuration being β) with anhydrous methanol (0.7914 g, 2.473×10^{-2} mol) in the presence or absence of an excess of the previous nucleophiles or in inert solvent results in the quantitative formation of methyl

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2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (from intermediates from reactions I and III; see Table I) and methyl 2,3,4-tri-*O*-benzyl-6-*O*-*p*-methoxybenzoyl-α-D-glucopyranoside (from reaction IV, Table I). The order of reactivity of these three reagents seems to follow sulfonium > ammonium > phosphonium. The sulfonium salt (reaction II, Table I) seems to be the most unstable and the most loose of the ion pairs for it gives lower steric purity in the methanolysis reaction. The phosphonium salt, on the other hand, required elevated temperature (Table I) in order to obtain complete conversion to the methyl glucopyranoside within the same reaction time. However, higher reaction temperature did not influence the stereoselective control of the product isolated from this intermediate.

At present further studies are being conducted in this laboratory to apply these and related nucleophilic reagents to the synthesis of a number of more complex glycosides.

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Steganacin and Steganangin, Novel Antileukemic Lignan Lactones from *Steganotaenia araliacea*[1-3]

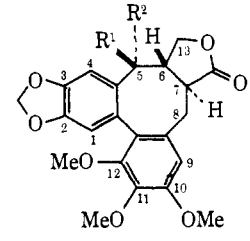
Sir:

An alcoholic extract of *Steganotaenia araliacea* Hochst.[4] was found to show significant activity *in vivo* against the P-388 leukemia in mice and *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[5] We report herein the isolation and structural elucidation of steganacin (**1**) and steganangin (**2**), two novel antileukemic[6] lignan lactones. These compounds and the companion lignans steganone (**5**) and steganol (**3**) appear to be the first reported bisbenzocyclooctadiene lactones.[7]

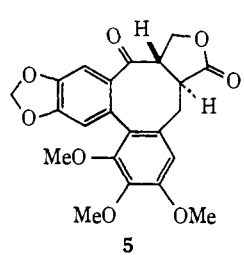
Fractionation of an ethanol extract, guided by assay against KB and P-388, revealed that the inhibitory activity was concentrated in the chloroform layer of a chloroform–water partition. The chloroform layer was partitioned between 10% aqueous methanol and Skellysolve B, and the 10% aqueous methanol layer was further partitioned between 20% aqueous methanol and carbon tetrachloride, which concentrated all of the activity in the final CCl_4 layer. Chromatography of this material on silica gel yielded a KB cytotoxic fraction (A) upon elution with 5% ether in benzene, an *in vivo* active fraction (B) on elution with 10% ether in benzene, and a further cytotoxic fraction (C) on elution with 100% ether. Preparative tlc of fraction A on Chromar 7GF plates with 10% ether in benzene gave two crystalline compounds. The first, steganangin (**2**, 0.1%), $C_{27}H_{28}O_9$, showed: mp 142.5–143°; $[\alpha]^{23}D$ −113° (*c* 0.72, $CHCl_3$); uv max (EtOH) 285 (ε 5180), 256 (ε 10,600), and 210 nm (end abs); ir (KBr) 5.66, 5.83, 5.88 (sh), 6.29, 8.20, 8.70, 8.78, and 9.65 μ; mass spectrum *m/e* 496 (M^+), 396, and 366; nmr (C_6D_6) τ 8.43 (3 H, m, angelate α methyl), 8.25 (3 H, br d, J = 7.6 Hz, angelate β methyl), 6.48, 6.42, 6.26 (9 H, 3 s, 3 OCH_3), 4.49, 4.48 (2 H, 2 d, AB quartet, J = 9 Hz, OCH_2O), 4.33 (1 H, m, angelate vinyl *H*), 4.27 (1 H, d, J = 10 Hz, 5-*H*), 3.68, 3.55 (2 H, 2 s, 1-*H*, 9-*H*), and 3.13 (1 H, s, 4-*H*). The second, steganone (**5**, 0.1%), $C_{22}H_{20}O_8$, showed: mp 155–156°; $[\alpha]^{23}D$ −202° (*c* 0.76, $CHCl_3$); uv max (EtOH) 317 (ε 5710), 276 (ε 9200), 238 (ε 27,600), and 210 nm (end abs); ir (KBr) 5.67, 6.00, 6.21, 6.30, 6.39, and 8.10 μ; mass spectrum *m/e* 412 (M^+), 398, 397, 328; nmr ($CDCl_3$) τ 6.46 (3 H, s, OCH_3), 6.17 (6 H, s, 2 OCH_3), 5.71 (1 H, q, B of ABX, 13-*H*), 5.58 (1 H, q, A of ABX, 13-*H*), 3.98 (2 H, br s, OCH_2O), 3.54, 3.44 (2 H, 2 s, 1-*H*, 9-*H*), and 2.55 (1 H, s, 4-*H*).

Preparative tlc of fraction B on Chromar 7GF using 10% ether in benzene gave steganacin (**1**, 0.4%): $C_{24}H_{24}O_9$; $[\alpha]^{23}D$ −114° (*c* 0.74, $CHCl_3$); uv max (EtOH) 285 (ε 5450), 255 (ε 10,700), and 210 nm (end abs); ir (KBr) 5.65, 5.78, 6.29, 8.10, 9.63, and 9.84 μ; mass spectrum *m/e* 456 (M^+), 396, 366; nmr ($CDCl_3$) τ 8.08 (3 H, s, $OCOCH_3$), 6.28, 6.14, 6.10 (9 H, 3 s, 3 OCH_3), 4.19 (1 H, br d, $J_{5,6}$ = 8 Hz, 5-*H*), 4.00 (2H, s, OCH_2O), 3.57, 3.42 (2 H, 2 s, 1-*H*, 9-*H*), and 3.11 (1 H, s, 4-*H*).

Preparative tlc of fraction C on silica gel plates with 1:1 ether–benzene gave steganol (**3**, 0.001%): $C_{22}H_{22}O_8$; $[\alpha]^{23}D$ −163° (*c* 0.87, $CHCl_3$); uv max (EtOH) 287 (ε 5600), 255 (ε 11,200), and 210 nm (end abs); ir (KBr) 2.90, 5.65, 6.28, 8.15, 9.62, and 9.85 μ; mass spectrum *m/e* 414 (M^+), 396, 330; nmr ($CDCl_3$) τ 6.27, 6.13, 6.09, (9 H, 3 s, 3 OCH_3), 3.98 (2 H, s, OCH_2O), 3.55, 3.43 (2 H, 2 s, 1-*H*, 9-*H*), and 3.22 (1 H, s, 4-*H*).



1, R^1 = OAc; R^2 = H



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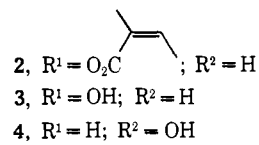
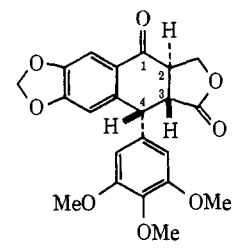


2, R^1 = O_2C ; R^2 = H
3, R^1 = OH; R^2 = H
4, R^1 = H; R^2 = OH



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From the spectral data it appeared that all four compounds were related and indeed it was shown that:

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(3) Supported by grants from the National Cancer Institute (CA-11718 and CA-11760) and American Cancer Society (T-275 and T-541), and a contract with the National Cancer Institute (NIH-NCI-C-71-2099).
(4) Wood of stems and stem bark were collected in Ethiopia in March 1971. We thank Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., for supplying the plant material.
(5) Cytotoxicity and *in vivo* activity were assayed as in *Cancer Chemother. Rep.*, **25**, 1 (1962).
(6) Steganacin and steganangin showed significant antileukemic activity against P-388 leukemia in the mouse, and cytotoxicity against a KB cell culture at 10^{-1}–10^{-3} μg/ml.
(7) N. K. Kochetkov, A. Khorlin, O. S. Chizov, and V. I. Sheichenko [*e.g.*, *Tetrahedron Lett.*, 730 (1961)] have described the only other representatives of the unusual bisbenzocyclooctadiene lignans.