counted for by the EFF calculations are operative for 1 in solution.

Experimental Section

NMR spectra were recorded on a Bruker WM-250 spectrometer. Temperature measurements were based on the chemical shift separation of an ethylene glycol sample, and utilization of the Van Geet relationship.[26] For the 2D spectra a sequence of three pulses $(90°-t_1-90°-t_m-90°)_n$, where t_m is the mixing time, was used as implemented in Bruker's program NOESY.AU. Thirty-two FIDs (each consisting of 16 scans of 128 data points) were accumulated. The FIDs were zero-filled to $128w$ in the F_1 dimension. The determination and the simulation of the molecular ion cluster was performed on a Kratos MS 50 RFA spectrometer. The high-resolution mass spectrum was obtained by the Midwest Center for Mass Spectrometry.

1,2,3,4,5,6,7,8-Octakis(dichloromethyl)anthracene (1). 1,2,3,4,5,6,7,8-Octamethylanthracene (90 mg, 0.31 mmol), prepared from 1,2,3,4,5,6,7,8-octamethyl-9,10-dihydroanthracene[27] according to the literature procedure,[28] was dissolved in 30 mL of CCl_4. The solution was heated under reflux and irradiated with a 150-W lamp while a slow stream of chlorine gas was introduced. After 16 h the reaction was stopped. The solid that had deposited on the gas inlet tube was collected to give 140 mg (64%) of essentially pure 1 (^{1}H NMR). The compound was recrystallized from 1,1,2,2-tetrachloroethane to afford transparent crystals, which turned immediately opaque upon filtration. The crystals were repeatedly washed with ether and dried by suction to yield a product, mp >300 °C, free of solvent. ^{1}H NMR (room temperature, $CDCl_2CDCl_2$) δ 7.53 (s, 1 H, br); 7.64 (s, 1 H, br); 8.21 (s, 1 H); 8.30 (s, 1 H); 8.33 (s, 1 H). High-resolution mass spectra (only the three most intense signals of the molecular ion cluster are given), m/z 839.5703 (839.5707 calcd for $C_{22}H_{10}{}^{35}Cl_{13}{}^{37}Cl_3$), 841.5674 (841.5676 calcd for $C_{22}H_{10}{}^{35}Cl_{12}{}^{37}Cl_4$), 843.5655 (843.5646 calcd for $C_{22}H_{10}{}^{35}Cl_{11}{}^{37}Cl_5$). The experimentally determined and calculated molecular ion clusters are in good agreement.

[9,10-$^2H_2$]-1,2,3,4,5,6,7,8-Octakis(dichloromethyl)-anthracene. A solution of 1 (10 mg) and CF_3COOD (1 mL, Aldrich, 99 atom % D) in 4 mL of hexane was stirred and heated under reflux for 20 h. After evaporation of the solvent, the ^{1}H NMR spectrum showed that the 9,10-positions were completely exchanged by deuterium. The deuteriated anthracene was chlorinated according to the procedure described for the unlabeled compound. ^{1}H NMR (room temperature, $CDCl_2CDCl_2$) δ 7.64 (s, 1 H); 8.21 (s, 1 H); 8.30 (s, 1 H); 8.33 (s, 1 H).

Acknowledgment. We thank the National Science Foundation (CHE-8510067) for support of this work.

Registry No. 1, 112947-60-1; 1,2,3,4,5,6,7,8-octamethylanthracene, 64094-28-6.

(26) Van Geet, A. L. *Anal. Chem.* **1968**, *40*, 2227; *Ibid.* **1970**, *42*, 679.
(27) Welch, C. M.; Smith, H. A. *J. Am. Chem. Soc.* **1951**, *73*, 4391.
(28) Backer, H. J.; Strating, J.; Huisman, L. H. H. *Recl. Trav. Chim. Pays-Bas* **1939**, *58*, 761.

A Regiocontrolled Synthesis of N^7- and N^9-Guanine Nucleosides

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The reaction of 2-O-acetylated and 2-O-benzoylated glycosides **3a,b/4a,b** with silylated N^2-acetylguanine **7** selectively gave N^7-guanine nucleosides **8a,b/9a,b** under kinetically controlled conditions ($SnCl_4/CH_3CN$, room temperature), whereas 2-O-benzoylated glycosides **3b/4b** selectively gave the isomeric N^9-guanine nucleosides **10b/11b** under thermodynamically controlled conditions (TMSOTf/$(CH_2Cl)_2$, reflux). Unambiguous assignment of nucleoside structure was accomplished after hydrolysis (NH_3/MeOH) of the initial products to the known nucleosides **8c**, **9c**, **10c**, and **11c** followed by ^{1}H and ^{13}C NMR spectral analysis. The described procedures provide the best method to date for the selective synthesis of either N^7- or N^9-guanine nucleosides from a common substrate.

As part of a program directed toward the synthesis of the nucleoside antibiotics amipurimycin (1) and miharamycin (2),[1] we required a method for the synthesis of N^9-pyranosyl-2-aminopurines from their corresponding glycosidic precursors. In this context a coupling procedure that involved the use of a readily available silylated guanine derivative in conjunction with a Lewis acid catalyst seemed most appropriate even though the regioselectivity of this reaction (ie. N^7- vs N^9-glycosylation) is not generally high with this base.[2-4] Once the desired N^9-nucleoside is obtained, however, modification of the guanine moiety to give the 2-aminopurine could then follow well established procedures.[5] Herein we report on work which has cul-

(1) Amipurimycin: Goto, T.; Toya, Y.; Ohgi, T.; Kondo, T. *Tetrahedron Lett.* **1982**, 1271. Miharamycin: Seto, H.; Koyama, M.; Ogino, H.; Tsuruoka, T.; Inouye, S.; Otake, N. *Ibid.* **1983**, 1805.

(2) For a review of developments in the area of nucleoside synthesis, see: Dekker, C. A.; Goodman, L. In *The Carbohydrates*, 2nd Ed.; Pigman, W., Horton, D., Herp, A., Eds.; Academic: New York, 1970. And more recently: Vorbrüggen, H. *NATO Adv. Study Inst. Ser., Ser. A* **1979**, *26*, 35.

(3) (a) Lichtenthaler, F. W.; Voss, P.; Heerd, A. *Tetrahedron Lett.* **1974**, 2141. (b) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234. (c) Morr, M. *Liebigs Ann. Chem.* **1982**, 666. The NMR data reported in this paper seems to indicate that a (2.5:1) mixture of N^9:N^7 regioisomers was obtained rather than the reported anomeric mixture of N^9-nucleosides—see Table I. (d) For a related "trans-nucleosidation", see: Azuma, T.; Isono, K. *Chem. Pharm. Bull.* **1977**, *25*, 3347.

(4) Previous approaches to nucleosides (and nucleotides) that also employed guanine derivatives directly for the coupling include: (a) Imai, K.; Nohara, A.; Honjo, M. *Chem. Pharm. Bull.* **1966**, *14*, 1377. (b) Nomura, H.; Suhara, I.; Uno, N. *Ibid.* **1967**, *15*, 1258. (c) Furukawa, Y.; Honjo, M. *Ibid.* **1968**, *16*, 1076. (d) Furukawa, Y.; Imai, K.; Honjo, M. *Tetrahedron Lett.* **1968**, 4655. (e) Suzaki, S.; Yamazaki, A.; Kamimura, A.; Mitsugi, K.; Kumashiro, I. *Chem. Pharm. Bull.* **1970**, *18*, 172. (f) Lee, W. W.; Martinez, A. P.; Goodman, L. *J. Org. Chem.* **1971**, *36*, 842. (g) Iwamura, H.; Miyakado, M.; Hashizume, T. *Carbohydr. Res.* **1973**, *27*, 149. (h) Hobbs, J. B.; Eckstein, F. *J. Org. Chem.* **1977**, *42*, 714.

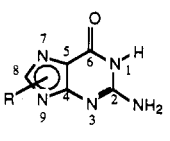
0022-3263/88/1953-1294$01.50/0

Table I. Regiocontrolled Glycosylation of Silylated N^2-Acetylguanine

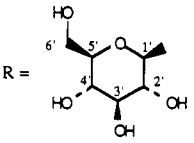
entry	substrate	procedure[a]	products[b]	N^7:N^9	yield,[c] %
1	**3a**	A	**8a/10a**	1:0	61
2	**3a**	B	**8a/10a**	1:2	72
3	**3b**	A	**8b/10b**	1:0	61
4	**3b**	B	**8b/10b**	1:8	56
5	**4a**	A	**9a/11a**	95:1	78
6	**4a**	B	**9a/11a**	1:3	81
7	**4b**	A	**9b/11b**	3:1	81
8	**4b**	B	**9b/11b**	1:6	79

[a] See Experimental Section for details. [b] Ratios determined from ^{1}H NMR integration of the completely deprotected nucleoside mixture ("c" series). [c] Combined yield of the protected nucleoside mixtures isolated by flash chromatography.

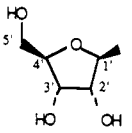
Table II. Selected ^{1}H and ^{13}C NMR Data for Unprotected Nucleosides[a]



R =



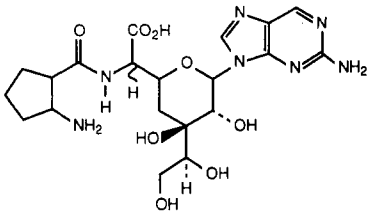
R =



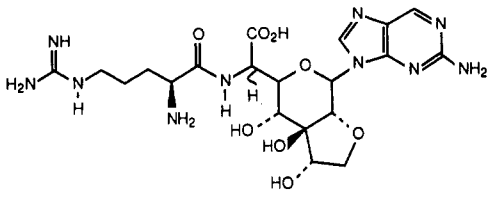
product	H-8	NH_2	C-4	C-5	C-8	C-1′
8c	8.14	6.12	159.86	108.06	142.08	84.72
9c	8.23	6.18	160.70	107.75	142.50	89.18
10c	7.84	6.50	151.61	116.30	135.69	82.09
11c	7.94	6.45	151.39	116.73	135.68	86.40

[a] All spectra were measured in DMSO-d_6 at ambient temperature; signals are reported in ppm (see Experimental Section).

minated in the first regiocontrolled synthesis of both N^7- and N^9-guanine nucleosides via glycosylation (Table I).

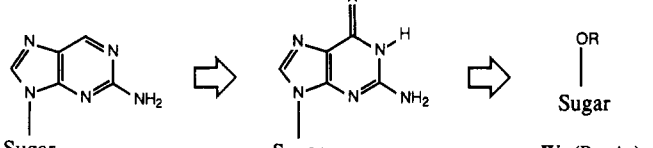


1



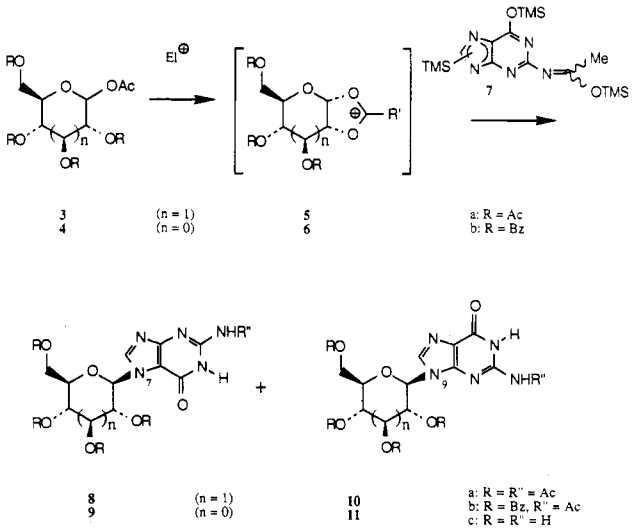
2

Strategy:



I II (X = S) III (X = O) IV (R = Ac) V (R = Me)

Initially we chose 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**3a**)[6] as a model substrate for glycosylation since targets **1** and **2** both incorporate a 4-deoxyglucopyranose substructure as well. Indeed, the reaction of **3a** with trisilylated N^2-acetylguanine **7** in acetonitrile proceeded at ambient temperature in the presence of tin tetrachloride (procedure A), but to our dismay resulted in exclusive formation of the *undesired* N^7-nucleoside **8a**. When the



same reaction was performed with trimethylsilyltriflate in refluxing 1,2-dichloroethane (procedure B), the N^9-isomer predominated only to the extent of (2:1). A variety of modifications (not shown) involving solvent composition, different catalysts, and nucleoside precomplexation were tried but did not increase the proportion of N^9-isomer significantly. Recalling that N^9-guanine nucleosides are thermodynamically favored over their N^7-isomers,[7] we reasoned that a 2-O-benzoylated sugar such as **3b** might be a better substrate since the intermediate cationic complex **5b** (R′ = C_6H_5) should be lower in energy (and equilibrium thus easier to attain) than **5a** (R′ = CH_3). Whereas the reaction of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose (**3b**)[6] with **7** under the conditions of procedure A again resulted in the exclusive formation of the (benzoylated) N^7-isomer **8b**, we were most pleased to find that application of procedure B to this same substrate resulted in the *selective* formation of the desired N^9-isomer **10b** as anticipated—with the overall N^9:N^7 ratio now (8:1)! *It is significant that these results define for the first time conditions by which both N^7- and N^9-guanine nucleosides may be synthesized in a completely regiocontrolled manner from the same substrate.*

Having succeeded in preparing both N^7- and N^9-*pyranosyl*guanines selectively, it was of interest to see if similar regiocontrol was possible in the furanose series as well. Once again, the combination of procedure A with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**4a**) resulted in selective formation of the N^7-nucleoside **9a** (N^7:N^9 = 95:1) whereas application of procedure B to 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**4b**) afforded the protected N^9-guanosine **11b** selectively (N^9:N^7 = 6:1). This latter result corroborated an earlier report by Vorbrüggen wherein he obtained a good yield of the hydrolysis product **11c** after recrystallization.[3b] We also observed that the rate of these furanose reactions is at least an order of magnitude faster than the pyranose series, a result consistent with

(5) Nair, V.; Young, D. A.; DeSilvia, R. Jr. *J. Org. Chem.* **1987**, *52*, 1344. Schaeffer, H. J.; Thomas, H. J. *J. Am. Chem. Soc.* **1958**, *80*, 4896. Fox, J. J.; Wempen, I.; Hampton, A.; Doerr, I. L. *Ibid.* **1958**, *80*, 1669.

(6) Compounds **3a** and **3b** were prepared from their corresponding methyl glycosides via Hudson's procedure: Hann, R. M.; Hudson, C. S. *J. Am. Chem. Soc.* **1934**, *56*, 2465.

(7) Miyaki, M.; Shimizu, B. *Chem. Pharm. Bull.* **1970**, *18*, 1446.

Table III. Pertinent NOE Difference Results with 9c[a]

irradiated proton	enhanced proton	% NOE
H-8	H-1′	14.9
H-8	H-3′	6.8
H-1′	H-8	20.8
H-3′	H-8	14.5
H-4′	H-1′	8.2
H-5′a	H-8	9.7
H-5′b	H-8	5.5

[a] This experiment was performed in D_2O + NaOD at ambient temperature on a Bruker MSL 400 NMR spectrometer using the NOE difference automation program (decoupler power = 10L). Difference spectra were obtained after manually zeroing the TMS signal of the TSP internal standard (see Experimental Section); only enhancements above a 5% threshold are reported.

rate-determining formation of the cyclic acyloxonium ions **5/6**.[8]

The peracylated nucleoside products were readily purified by flash chromatography and in some cases by crystallization as well. Unambiguous assignment of nucleoside structure was best accomplished after hydrolysis (NH_3/MeOH) of the initial products to give the known nucleosides **8c**, **9c**, **10c**, and **11c**. In keeping with observations reported for a series of N-alkylated purines and related nucleosides,[9] the ^1H and ^{13}C NMR spectra obtained for these compounds were quite indicative regarding the point of sugar attachment to the guanine moiety (Table II). For instance the H-8 proton signal and the C-4, C-8, and C-1′ carbon signals of the N^7-nucleosides **8c** and **9c** are shifted downfield, whereas the NH_2 proton signal and the C-5 signal are shifted upfield relative to the corresponding resonances of the N^9-isomers **10c** and **11c**. Parenthetically we note that all of the peracylated N^7-nucleosides were consistently more mobile than their N^9-isomers when analyzed by normal-phase TLC on silica gel (see Experimental Section).

The β-anomeric configuration shown for the pyranosylguanines **8a–c** and **10a–c** was readily deduced from the trans-diaxial coupling (>9 Hz) observed between H-1′ and H-2′ in the ^1H NMR spectra of **8a,c** and **10a,c**. Such reliance on vicinal coupling constants is not always warranted with furanose derivatives due to conformational fluctuations. In the case of the N^9-furanosylguanines **11a–c**, the β-configuration was confirmed by correlation of **11c** with an authentic sample of guanosine. The N^7-isomers **9a–c** were unambiguously shown to possess the β-anomeric configuration from a series of NOE experiments with **9c** that showed significant enhancement of H-8 upon irradiation of H-3′ and H-5′ respectively as well as enhancment of H-1′ when H-4′ was irradiated (Table III).

In conclusion, we have defined reaction parameters that allow regioselective synthesis of both N^7- and N^9-guanine nucleosides via Lewis acid mediated coupling of acylated sugars and a silylated base. Conditions which favor kinetic control lead to formation of the N^7-isomers whereas the N^9-isomers result from conditions that ensure thermodynamic control. The exact mechanism of these (and related) nucleoside-forming reactions appears to be complex and is still incompletely understood.[10] Even though our initial rationale was based on thermodynamic considerations (vide supra), there may indeed be a kinetic component responsible for the selective formation of the N^9-nucleosides as well. In such a case the transition states for both **5 + 7 → 8 + 10** and **6 + 7 → 9 + 11** would be "later" with the 2-benzoates **5b/6b** relative to the 2-acetates **5a/6a** and thus also result in accentuated N^9-selectivity. In any event the described procedures provide the best method to date for the synthesis of either N^7- or N^9-guanine nucleosides and also illustrate the importance of sugar-protecting groups for the fine-tuning of these coupling reactions.[11]

Experimental Section

All coupling reactions were performed under a nitrogen atmosphere. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarimeter and are the average of at least four measurements. ^1H NMR spectra were recorded at 200 MHz and ^{13}C NMR at 50.4 MHz with a Varian XL 200 spectrometer using residual $CHCl_3$ and DMSO as well as the water-soluble 3-(trimethylsilyl)propionic acid, sodium salt (TSP, Aldrich) as internal standards. The ^{13}C assignments were based on both APT (attached proton test)[12] experiments and ^1H-coupled spectra. The APT results are indicated as "+" or "–" depending on the phase of the signal. IR spectra were recorded on a Perkin-Elmer 1420 spectrophotometer. UV spectra were recorded on a Cary 2300 spectrophotometer. Combustion analyses were performed on TLC homogeneous or recrystallized samples by Galbraith Labs, Inc., and the "exact" degree of hydration was determined computationally.

Acetonitrile and 1,2-dichloroethane were distilled from CaH_2 and P_2O_5, respectively, while trimethylsilyl triflate and tin tetrachloride were distilled under N_2 atmosphere just prior to use. 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**4a**) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**4b**) were purchased from Aldrich Chem. Co. Trisilylated N^2-acetylguanine **7** was prepared from N^2-acetylguanine following the published procedure.[8d] TLC analysis was performed on Merck silica gel 60 F-254 plates and visualized first by UV illumination and then by charring with 5% anisaldehyde in EtOH–HOAc–H_2SO_4 (95:5:1). The TLC solvent systems employed were (A) EtOAc–hexanes (1:1), (B) ethyl acetate, and (C) EtOAc–MeOH (14:1).

Procedure A. To a 0.15 M solution of **3a,b** or **4a,b** in acetonitrile was added 1.3–1.7 equiv of a 4 M solution of trisilylated *N*-acetylguanine **7** in acetonitrile followed by 4.5–5.5 equiv of neat tin tetrachloride. The homogeneous mixture was stirred at ambient temperature for 16 h in the case of **3a,b** and 4 h in the case of **4a,b**, at which time TLC indicated disappearence of most of **3a,b** and complete consumption of **4a,b**. At this point the reaction mixture was diluted with methylene chloride (80 mL) and washed with saturated $NaHCO_3$ solution (2 × 25 mL) and then brine (2 × 25 mL). The organic layer was dried with $MgSO_4$, filtered, concentrated, and flash chromatographed on silica gel by using ethyl acetate and 3% MeOH–EtOAc. Where possible, analytical samples were purified by recrystallization from hot MeOH.

Procedure B. To a 0.15 M solution of **3a,b** or **4a,b** in 1,2-dichloroethane was added 1.3–1.7 equiv of a 4 M solution of **7** in 1,2-dichloroethane followed by 2.1–2.4 equiv of a 0.4 M solution

(8) This difference in rate is probably due to a combination of factors including the favorable 1,2-trans relationship in substrates **4a,b** (vs 1,2-cis in **3a,b**) as well as the increased stability of the five-membered cyclic acyloxonium ion **6** (over the six-membered analogue **5**). For an early review of the role of cyclic acyloxonium ions in the carbohydrate field, see: Lemieux, R. *Adv. Carbohydr. Chem.* **1954**, *9*, 1.

(9) Kjellberg, J.; Johansson, N. G. *Tetrahedron* **1986**, *42*, 6541; Chenon, M.-T.; Pugmire, R. J.; Grant, D. M.; Panzica, R. P.; Townsend, L. B. *J. Am. Chem. Soc.* **1975**, *97*, 4627. This method for determination of purine regiochemistry based on NMR appears to be quite reliable and is more convenient than the "traditional" UV studies.

(10) (a) For a "mechanistic overview" of nucleoside synthesis via condensation reactions, see: Watanabe, K. A.; Hollenberg, D. H.; Fox, J. J. *J. Carbohydr., Nucleosides, Nucleotides* **1974**, *1*, 1. (b) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256. The effect of competitive σ-complex formation between the Lewis acid catalyst and silylated base during the nucleoside-forming reaction is discussed in this paper.

(11) Prior to this work, a selective but somewhat lengthy synthesis of N^7-nucleosides had been accomplished via the elaboration of imidazole nucleosides: Rousseau, R. J.; Robins, R. K.; Townsend, L. B. *J. Am. Chem. Soc.* **1968**, *90*, 2661.

(12) For more details about the "attached proton test" (APT), see: *XL-Series NMR Spectrometer System. Advanced Operation*, Publication No. 87-146-006, Rev. A383; Varian Instrument Division: Palo Alto, CA, 1983; pp 2–29. LeCocq, C.; Lallemand, J.-Y. *J. Chem. Soc., Chem. Commun.* **1981**, 150.

of trimethylsilyl triflate. The mixture was then heated under reflux (bath T = 115 °C) for 16 h in the case of **3a,b** and 1.5 h in the case of **4a,b**, at which time TLC indicated disappearence of most of **3a,b** and complete consumption of **4a,b**. The reaction mixture was cooled and worked up as described in procedure A.

Deacylation. The protected nucleosides **8a,b/10a,b** and **9a,b/11a,b** were dissolved in NH_3/MeOH and kept at ambient temperature for 42 h. The mixture was concentrated and the residue dissolved in H_2O and extracted with methylene chloride to remove $RCONH_2$ byproducts. Concentration of the aqueous layer afforded the crude nucleosides (ca. 85% yield), which were examined by NMR to determine N^7:N^9 ratios prior to recrystallization from hot H_2O.

N^2-Acetyl-7-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)guanine (8a): R_f 0.58 (solvent system C); mp 295–296 °C (from MeOH) [lit.[4g] mp 299–301 °C]; $[\alpha]_D$ −38.9° (*c* 1.14, $CHCl_3$); UV (MeOH) λ_{max} 262 nm (ε 9158), 282 (7271) [lit.[4g] (MeOH) λ 263 nm (ε 13500), 283 (sh) (10100)]; IR ($CHCl_3$) 1760, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 55 °C) δ 10.50 (br s, H, disappears with D_2O exchange), 8.01 (s, H), 6.10 (d, J = 9.60 Hz, H), 5.62 (t, J = 9.3 Hz, H), 5.41 (t, J = 9.25 Hz, H), 5.26 (t, J = 9.6 Hz, H), 4.30–3.98 (m, 3 H), 2.37 (s, 3 H), 2.05, 2.04, 2.00, 1.84 (all s, each 3 H), 1.50 (s, 2 H, disappears with D_2O exchange). Anal. Calcd for $C_{21}H_{25}O_{11}N_5 \cdot 0.66\ H_2O$: C, 47.10; H, 4.96; N, 13.08. Found: C, 47.50; H, 4.91; N, 12.91.

***N*-Acetyl-7-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glucopyranosyl)guanine (8b)**: R_f 0.68 (solvent system C); mp 283–284 °C dec (from MeOH); $[\alpha]_D$ +33° (*c* 0.54, $CHCl_3$); UV (MeOH) λ_{max} 228 nm (ε 23427), 262 (6600), 282 (5938), 295 (3297), [lit.[7] (EtOH) λ 225.5 nm (ε 52400), 265.5 (16500), 275 (sh) (15300), 283 (sh) (13300)]; IR ($CHCl_3$) 1735, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 55 °C) δ 10.45 (br s, H, disappears with D_2O exchange), 8.17 (s, H), 8.03–7.13 (m, 20 H), 6.46 (m, H), 6.12 (m, 2 H), 5.89 (m, H), 4.63–4.46 (m, 3 H), 2.29 (s, 3 H), 1.52 (br s, 6 H, disappears with D_2O exchange). Anal. Calcd for $C_{41}H_{33}N_5O_{11} \cdot 3.3\ H_2O$: C, 59.25; H, 4.80; N, 8.43. Found: C, 58.76; H, 4.26; H, 8.19.

7-β-D-Glucopyranosylguanine (8c): mp 297–300 °C (from H_2O) [lit.[4g] mp 299–301 °C]; $[\alpha]_D$ −17° (*c* 0.20, 0.1 N NaOH), [lit.[7] $[\alpha]_D$ −17° (*c* 1.0, 0.1 N NaOH)]; UV (H_2O) λ_{max} 285 nm (ε 7200), (0.1 N NaOH) 283 (2405), (0.1 N HCl) 259 (4373), [lit.[4g] (H_2O) λ 287 nm (ε 7500), (0.1 N NaOH) 284 (6290), (0.1 N HCl) 256 (7910)]; IR (KBr) 3300, 3140, 1695, 1640 cm^{-1}; ^1H NMR (DMSO-d_6, room temperature) δ 8.14 (s, H), 6.12 (s, 2 H), 5.52 (d, J = 9.3 Hz, H), 5.29 (d, J = 5.7 Hz, H), 5.22 (br s, H), 5.07 (d, J = 4.9 Hz, H), 4.52 (t, J = 3.5 Hz, H), 3.4–3.8 (m, 2 H), (D_2O, NaOD, room temperature) δ 8.17 (s, H), 5.87 (d, J = 9.37 Hz, H), 4.08 (t, J = 8.9 Hz, H), 3.89 (t, J = 13.1 Hz, H), 3.5–3.80 (m, 4 H); ^{13}C NMR (DMSO-d_6) δ 159.86 (s, +, C-4), 154.11 (s, +, C-6), 152.71 (s, +, C-2), 142.08 (d, −, J = 213.3 Hz, C-8), 108.06 (s, +, C-5), 84.72 (d, −, J = 156 Hz, C-1′), 79.73 (d, −, J = 136.2 Hz, C-2′), 77.16 (d, −, J = 127.8 Hz, C-3′), 71.67 (d, −, J = 102.9 Hz, C-4′), 69.52 (d, −, J = 109.3 Hz, C-5′), 60.85 (t, +, J = 141 Hz, C-6′). Anal. Calcd for $C_{11}H_{15}N_5O_6 \cdot 1.25\ H_2O$: C, 39.35; H, 5.25; N, 20.86. Found: C, 39.39; H, 4.67; N, 20.60.

N^2-Acetyl-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)guanine (9a): R_f 0.33 (solvent system B); mp 126–129 °C; $[\alpha]_D$ +39° (*c* 0.60, $CHCl_3$); UV (MeOH): λ_{max} 208 nm (ε 40071), 259 (23195), 278 (16664), [lit.[3d] (EtOH) λ 255 nm, 263, 285 (sh)]; IR ($CHCl_3$) 1750, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 55 °C) δ 10.36 (br, s, H, disappears with D_2O exchange), 8.06 (s, H), 6.33 (d, J = 4.6 Hz, H), 5.75 (dd, J = 5.5 and 4.5 Hz, H), 5.47 (m, H), 4.42 (br s, 3 H), 2.37 (s, 3 H), 2.11 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 3 H), 1.50 (br s, 2 H, disappears with D_2O exchange). Anal. Calcd for $C_{18}H_{21}N_5O_9 \cdot 0.5\ H_2O$: C, 46.96; H, 4.82; N, 15.21. Found: C, 47.15; H, 5.19; N, 14.68.

7-β-D-Ribofuranosylguanine (9c): mp 298 °C dec (from H_2O) [lit.[4g] mp >300 °C]; $[\alpha]_D$ −8.8° (*c* 0.32, 0.1 N NaOH); UV (H_2O) λ_{max} 286 nm (ε 9248), 240 (7940), (0.1 N NaOH) 282 (5451), 237 (6424), (0.1 N HCl) 251 (8503), 270 (sh) (6287), [lit.[4g] (H_2O) λ 287 nm (ε 7490), (0.1 N NaOH) 284 (6290), (0.1 N HCl) 251 (8820)]; IR (KBr) 3220, 3160, 1650, 1400 cm^{-1}; ^1H NMR (DMSO-d_6, room temperature) δ 8.23 (s, H), 6.18 (s, 2 H), 5.96 (d, J = 5.7 Hz, H), 5.37 (d, J = 6.1 Hz, H), 5.12 (d, J = 4.9 Hz, H), 5.03 (d, J = 5.1 Hz, H), 4.35 (dd, J = 11.1 and 5.0 Hz, H), 4.05 (dd, J = 8.4 and 4.3 Hz, H), 3.87 (dd, J = 6.4 and 2.6 Hz, H), 3.51 (m, 3 H), (D_2O/NaOD, room temperature) δ 8.17 (s, H), 6.07 (d, J = 5.8 Hz, H), 4.53 (t, J = 5.5 Hz, H), 4.20 (t, J = 4.2 Hz, H), 4.14 (m, H), 3.90 (dd, J = 12.5 and 3.0 Hz, H), 3.78 (dd, J = 12.5 and 4.5 Hz, H); ^{13}C NMR (DMSO-d_6, (room temperature) δ 160.7 (s, +, C-4), 154.5 (s, +, C-6), 153 (s, +, C-2), 142.5 (d, J = 209.7 Hz, C-8), 107.75 (s, +, C-5), 89.18 (d, −, J = 168.7 Hz, C-1′), 85.27 (d, −, J = 152.7 Hz, C-2′), 74.5 (d, −, J = 149.2 Hz, C-3′), 69.79 (d, −, J = 150.3 Hz, C-4′), 61.22 (t, +, J = 140 Hz, C-5′). Anal. Calcd for $C_{10}H_{13}N_5O_5 \cdot 1.45\ H_2O$: C, 38.82; H, 5.18; N, 22.64. Found: C, 39.19; H, 4.97; N, 22.10.

N^2-Acetyl-9-(2′,3′,4′,6′,-tetra-*O*-acetyl-β-D-glucopyranosyl)guanine (10a): R_f 0.55 (solvent system C); mp 295–298 °C [lit.[4g] mp 298–300 °C]; $[\alpha]_D$ −29.7° (*c* 1.00, $CHCl_3$); UV (EtOH) λ_{max} 254 nm (ε 8718), 282 (sh) (6217), [lit.[4g] (EtOH) λ 256 nm (ε 15800), 282 (sh) (11100)]; IR ($CHCl_3$) 1760, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 55 °C) δ 8.77 (s, H), 7.77 (s, H), 5.75–5.57 (m, 2 H), 5.39 (t, J = 9.5 Hz, H), 5.24 (t, J = 9.6 Hz, H), 4.27 (dd, J = 12.9 and 5.1 Hz, H), 4.16 (dd, J = 12.9 and 3.8 Hz, H), 4.05–3.85 (m, H), 2.82 (s, 3 H), 2.02 (s, 3 H), 2.04 (s, 3 H), 2.00 (s, 3 H), 1.80 (s, 3 H).

N^2-Acetyl-9-(2′,3′,4′,6′,-tetra-*O*-benzoyl-β-D-glucopyranosyl)guanine (10b): R_f 0.66 (solvent system C); mp 250 °C, dec starts at 215 °C; $[\alpha]_D$ +0.9°, $[\alpha]_{578}$ +10.5°, $[\alpha]_{546}$ +9.5°, $[\alpha]_{436}$ +15°, $[\alpha]_{366}$ +34° (*c* 0.44, $CHCl_3$); UV (MeOH) λ 230 nm (ε 54885), 260 (16140), 275 (14035), 282 (12280); IR ($CHCl_3$) 1725, 1695 (sh) cm^{-1}; ^1H NMR ($CDCl_3$, 55 °C) δ 8.25 (br s, H, disappears with D_2O exchange), 7.1–7.97 (m, 24 H), 6.12 (m, 2 H), 5.88 (m, 2 H), 4.4–4.7 (m, 3 H), 2.2 (s, 3 H), 1.54 (br s, 2 H, disappears with D_2O exchange). Anal. Calcd for $C_{41}H_{33}N_5O_{11} \cdot 1.5\ H_2O$: C, 61.65; H, 4.54; N, 8.78. Found: C, 61.9; H, 4.4; N, 8.41.

9-β-D-Glucopyranosylguanine (10c): 285–289 °C (from H_2O) [lit.[4g] mp 289–292 °C]; $[\alpha]_D$ −47° (*c* 0.21, 0.1 N NaOH); UV (H_2O) λ_{max} 246 nm (ε 12914), 271 (8878), (0.1 N NaOH) 265 (7925), (0.1 N HCl) 273 (sh) (8200), [lit.[4g] (H_2O) λ 253 nm (ε 13910), 270 (sh) (9730), (0.1 N NaOH) 263 (br) (11340), (0.1 N HCl) 257 (12760), 275 (sh) (8860)]; IR (KBr) 3320, 3130, 1645, 1395 cm^{-1}; ^1H NMR (DMSO-d_6, room temperature) δ 7.84 (s, H), 6.5 (br s, 2 H), 5.29 (d, J = 5.8 Hz, H), 5.25 (br m, H), 5.14 (d, J = 9.5 Hz, H), 5.1 (br m, H), 4.6 (t, J = 5.8 Hz, H), 3.4–3.8 (m, 2 H), (D_2O/NaOD room temperature) δ 7.89 (s, H), 5.40 J = 9.4 Hz, H), 4.13 (t, J = 8.6 Hz, H), 3.88 (t, J = 13.6 Hz, H), 3.5–3.8 (m, 4 H); ^{13}C NMR (DMSO-d_6, room temperature) δ 156.74 (s, +, C-6), 153.63 (s, +, C-2), 151.61 (s, +, C-4), 135.69 (d, J = 213.3 Hz, C-8), 116.3 (s, +, C-5), 82.09 (d, −, J = 108.9 Hz, C-1′), 80.1 (d, −, J = 90.9 Hz, C-2′), 77.35 (d, −, J = 128.9 Hz, C-3′), 71.25 (d, −, J = 142.1 Hz, C-4′), 69.66 (d, −, J = 145.5 Hz, C-5′), 60.87 (t, +, J = 140 Hz, C-6′). Anal. Calcd for $C_{11}H_{15}N_5O_6 \cdot 1.5\ H_2O$: C, 38.83; H, 5.33; N, 20.58. Found: C, 38.79; H, 5.10; N, 20.93.

***N*-²-Acetyl-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)guanine (11a)**: R_f 0.24 (solvent system B); mp 125–128 °C; $[\alpha]_D$ −63° (*c* 0.44, $CHCl_3$); ^1H NMR ($CDCl_3$, 55 °C) δ 8.89 (br s, H, disappears with D_2O exchange), 7.66 (s, H), 5.91 (m, 2 H), 5.76 (m, H), 4.66 (m, H), 4.45 (m, 3 H), 2.29 (s, 3 H), 2.13 (s, 3 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 1.51 (br s, 3.75 H, disappears with D_2O exchange).

N^2-Acetyl-9-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)guanine (11b): R_f 0.58 (solvent system B); mp 136–138 °C, (dec begins at 128 °C; $[\alpha]_D$ −61.3° (*c* 1.06, $CHCl_3$); UV (MeOH) λ_{max} 248 nm (ε 16504), 266 (39144), 269 (12644), [lit.[7] (EtOH) λ 231 nm (ε 46500), 252 (20600), 260 (19400), 275 (15100), 282 (14700)]; IR ($CHCl_3$) 1720, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 55 °C) δ 10.05 (br s, H, disappears with D_2O exchange), 7.2–8.1 (m, 16 H), 6.2–6.38 (m, 2 H), 6.42 (d, J = 5.4 Hz, H), 5.03 (dd, J = 10.99 and 4.28 Hz, H), 4.77 (m, 2 H), 2.28 (s, 3 H), 1.82 (br s, 3.5 H, disappears with D_2O exchange). Anal. Calcd for $C_{33}H_{27}N_5O_9 \cdot 0.65\ H_2O$: C, 61.04; H, 4.39; N, 10.79. Found: C, 61.51; H, 4.23; N, 10.10.

9-β-D-Ribofuranosylguanine (11c): mp 258 °C dec (from H_2O) [lit.[4g] mp 248–250 °C dec]; $[\alpha]_D$ −56° (*c* 0.48, 0.1 N NaOH), [lit.[3d] $[\alpha]_D$ −71.9° (*c* 1.078, 0.1 N NaOH)]; UV (H_2O) λ_{max} 253 nm (ε 13910), 270 (shoulder) (9730), (0.1 N HCl) 257 (12760), 275 (shoulder) (8860), (0.1 N NaOH) 263 (11340), [lit.[4g] (H_2O) λ 254 nm (ε 14150), 270 (sh) (9980), (0.1 N HCl) 257 (12700), 276 (sh) (8830), (0.1 N NaOH) 263 (br) (11110)]; IR (KBr): 3400, 3120, 1690, 1390 cm^{-1}; ^1H NMR (DMSO-d_6, room temperature) δ 10.62 (br s, H), 7.94 (s, H), 6.45 (br s, 2 H), 5.69 (d, J = 6.1 Hz, H), 5.40 (d, J = 6.0 Hz, H), 5.13 (d, J = 4.8, H), 5.04 (d, J = 5.5, H), 4.39 (dd, J = 11.0 and 5.9 Hz, H), 4.07 (dd, J = 8.3 and 4.5, H), 3.85

(dd, J = 7.1 and 4.0 Hz, H), 3.56 (m, 3 H), (D_2O/NaOD, room temperature δ 7.86 (s, H), 5.7 (d, J = 6.5 Hz, H), 4.68 (m, H), 4.20 (m, 2 H), 3.79 (dd, J = 12.6 and 3.4 Hz, H), 3.89 (dd, J = 12.6 and 2.9 Hz, H); ^{13}C NMR (DMSO- d_6, room temperature) δ 156.85 (s, +, C-6), 153.72 (s, +, C-2), 151.39 (s, +, C-4), 135.68 (d, J = 214.5 Hz, C-8), 116.73 (s, +, C-5), 86.4 (d, –, J = 164.4 Hz, C-1′), 85.27 (d, – J = 148.3 Hz, C-2′), 73.77 (d, –, J = 148 Hz, C-3′), 70.45 (d, –, J = 150.4 Hz, C-4′), 60.47 (t, +, J = 141 Hz, C-5′). Anal. Calcd for $C_{10}H_{13}N_5O_5 \cdot 2H_2O$: C, 37.62; H, 5.37; N, 21.93. Found: C, 37.61; H, 4.92; N, 21.53.

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Scytonemin A, a Novel Calcium Antagonist from a Blue-Green Alga

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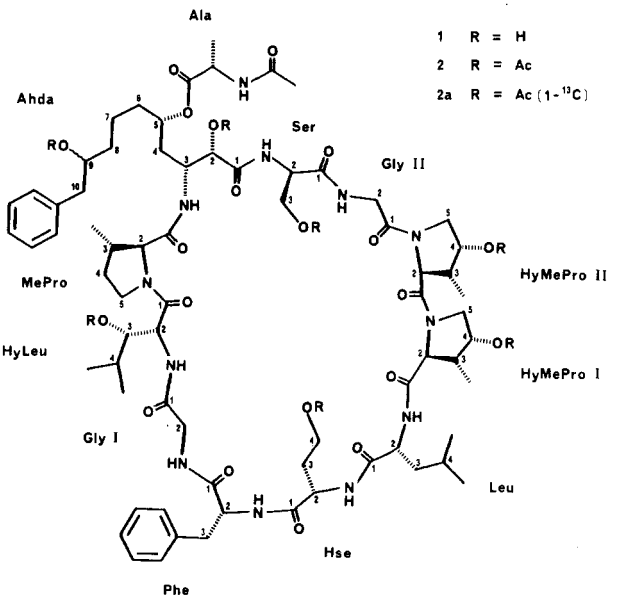
A novel cyclic peptide, scytonemin A, possessing potent calcium antagonistic properties is a major metabolite of the cultured cyanophyte *Scytonema* sp. (strain U-3-3). Vigorous acid hydrolysis of scytonemin A leads to L-alanine, 2 equiv of glycine, L-homoserine (Hse), D-(2*R*,3*S*)-*threo*-3-hydroxyleucine (HyLeu), D-leucine, D-serine, L-(2*S*,3*S*)-*trans*-3-methylproline (MePro), 2 equiv of L-(2*S*,3*R*,4*R*)-4-hydroxy-3-methylproline (HyMePro), D-phenylalanine, and (2*S*,3*R*,5*S*)-3-amino-2,5,9-trihydroxy-10-phenyldecanoic acid (Ahda). Mild acid hydrolysis results in predominantly two acyclic peptides, viz. Ser-Gly-HyMePro-HyMePro-Leu-Hse and Phe-Gly-HyLeu-MePro-Ahda. Still milder hydrolysis results in selective cleavage of the homoseryl amide bond in scytonemin A to give an acyclic peptide, Phe-Gly-HyLeu-MePro-Ahda-Ser-Gly-HyMePro-HyMePro-Leu-Hse, with an *N*-acetylalanyl unit attached via an ester linkage to C-5 of Ahda and a homoseryl lactone unit at the carboxyl terminus. State-of-the-art NMR and MS techniques have been used to determine the total structures of scytonemin A and the degradation products.

The blue-green algae have until recently been largely overlooked as a source of new pharmaceuticals and agrochemicals. Malyngolide,[1] majusculamide C,[2] cyanobacterin,[3] hapalindole A,[4] and scytophycins A and B[5] are examples of bioactive agents from this ubiquitous group of prokaryotic organisms which have already been described. We report here the isolation and structure elucidation of an unusual cyclic peptide, scytonemin A (1), from a *Scytonema* sp. (strain U-3-3) (Scytonemataceae)[6] which possesses potent calcium antagonistic properties.[7]



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(6) U-3-3 was originally identified as a *Plectonema* sp. (Geitler, L. In *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*; Rabenhorst, L., Ed.; Koeltz Scientific Books: Wiesbaden, 1985 (reprint); Vol. 14.] since heterocysts could not be detected in the original algal isolate or any subculture grown under the conditions described in ref 4b. When U-3-3 was grown on ^{15}N-enriched nitrate, however, heterocysts developed in the cultured alga which meant the cyanophyte was a *Scytonema* sp. Interestingly heterocysts disappeared when the heterocystous U-3-3 was regrown on ^{14}N-nitrate.

(7) On atria calcium antagonistic effects were observed at 5 μg/mL but not at 2.5 μg/mL; by comparison diltiazem was active at 2.5 μg/mL. On rat portal vein calcium blocking was observed at 20 μg/mL but not at 10 μg/mL; diltiazem showed activity at 0.5 μg/mL. Scytonemin A showed weak activity against a wide spectrum of bacteria and fungi; for example, activity was observed against *Mycobacterium ranae* at 1 μg/mL (MIC) but not at 0.5 μg/mL (MIC). By comparison gentamycin showed activity against *M. ranae* at 0.5 μg/mL (MIC). Weak antiprotozoal activity was noted against *Trichomonas vaginalis* and *Tritrichomonas foetus* at 1.56 and 3.12 μg/mL, respectively; metronidazole showed activity at 0.78 μg/mL. Some activity was observed against coccidia (*Eimeria tenella*) at 2.5 μg/mL but not at 1.25 μg/mL. Scytonemin A was mildly cytotoxic (IC50 = 2.9 μg/mL against CCRF-CEM).

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