Gabosines L, N and O: New Carba-Sugars from *Streptomyces* with DNA-Binding Properties

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In addition to the known gabosines A (**4**), B (**5**) and C (**6**), three new gabosines L (**1**), N (**2**) and O (**3**) were detected by chemical screening as secondary metabolites of *Streptomyces* (strains GT 041230, GT 051024 and S 1096). The constitutions of **1**, **2** and **3** were established by spectroscopic techniques and derivatization reactions. The absolute stereochemistry of **1** and **2** was determined by Helmchen's method and has been verified in the case of gabosine N (**2**) by X-ray analysis. The DNA-binding properties of the gabosines were investigated and analyzed by binding studies using a recently developed thin-layer chromatography technique (bimolecular-chemical screening).

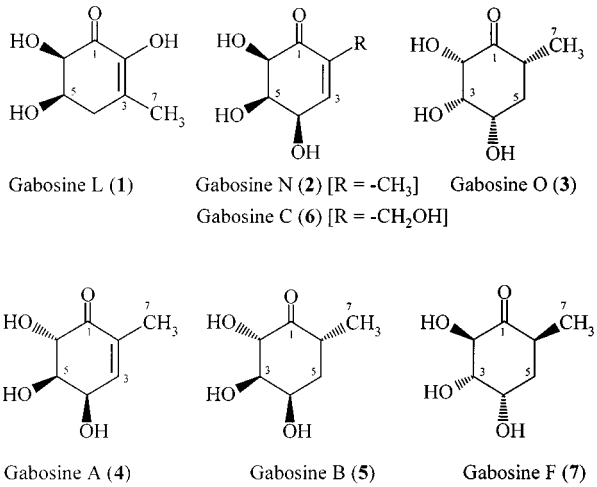
Introduction

Chemical screening[1] was found to be an efficient approach for the discovery of predominantly new secondary metabolites from natural sources. Application of this method to various new *Streptomyces* isolates led to the detection and isolation of a series of new carba-sugars named gabosines[2–4] for which, as yet, no biological activity could be found. In this article we describe the isolation and structure elucidation of three new gabosines named gabosine L (**1**), N (**2**) and O (**3**). In the course of our work on the gabosines and the so-called biomolecular screening,[5] we surprisingly discovered weak DNA-binding properties of **1**, **2** and **3** which led us additionally to examine other gabosines for DNA binding.

Results and Discussion

In our screening routine[1] the prepared extracts of the *Streptomyces* strains GT 041230 and GT 051024 showed striking brown spots on silica gel HPTLC plates on spraying with anisaldehyde/H_2SO_4 [R_f = 0.25, $CHCl_3$/MeOH, 9:1, (**2**) from GT 041230; R_f = 0.26 (**3**) and 0.27 (**5**) from GT 051024]. In order to isolate these metabolites, each of the two strains was cultivated in a fermentor (50 L for GT 051024, 25 L for GT 041230) containing a soybean meal/mannitol medium (180 rpm, aeration: 4 L/min, 28°C, 4 d). From the culture filtrates **2**, **3** and **5** were extracted with methanol, and purified by column chromatography on silica gel ($CHCl_3$/MeOH gradients) and gel permeation chromatography on Sephadex LH-20 (methanol) to yield 4.0 mg/L of **2**, 0.02 mg/L of **3**, and 0.8 mg/L of **5**.

The third strain *Streptomyces cellulosae* S 1096 was known to produce the gabosines A (**4**), B (**5**) and C (**6**).[2] Compound **6** is identical to the antibiotic KD 16-U1.[3][4] During a more detailed examination of the metabolic finger-print analyzed by TLC, two new gabosines L (**1**) and N (**2**) were detected and isolated. This was accomplished by cultivation of strain S 1096 in a 10-L fermentor (200 rpm, aeration: 1 vvm, 30°C, 4 d) containing a medium of rolled oats (2%, cooked and filtered). The culture filtrate was lyophilized and then extracted with methanol. The crude product was chromatographed on silica gel ($CHCl_3$/MeOH, 7:3). Further purification on Sephadex LH-20 with methanol and acetone gave 25 mg/L of **1** [R_f = 0.20, $CHCl_3$/MeOH, 9:1], 15 mg/L of **2**, 86 mg/L of **4** [R_f = 0.35], 205 mg/L of **5** [R_f = 0.38], and 49 mg/L of **6** [R_f = 0.10], respectively.



Scheme 1. Structures of selected gabosines

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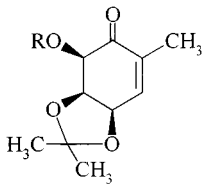
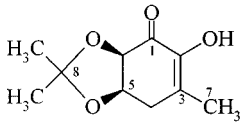
Gabosine L (1)

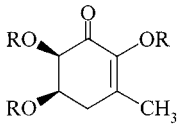
The molecular formula $C_7H_{10}O_4$ (M^+: 158.0579) of **1** was established from high-resolution EI mass spectrum. An IR absorption band at 1679 cm^{-1} showed the presence of an α,β-unsaturated carbonyl group which was confirmed by the ^{13}C-NMR signals (CD_3OD) at δ = 195.0 (s, C-1), 143.8 (s, C-3) and 127.4 (s, C-2). Furthermore, two methine groups linked to oxygen atoms [δ = 71.6 (d, C-5) and 76.5 (d, C-6)], one methylene group [δ = 37.0 (t, C-4)] and one methyl group [δ = 17.0 (q, C-7)] were detected. The constitution of gabosine L (**1**) i.e. 2,5,6-trihydroxy-3-methyl-2-cyclohexenone was proved by a detailed NMR data examination from ^1H-^1H-COSY, HSQC and HMBC experiments. The *cis* configuration of the two adjacent hydroxy groups was confirmed from the 5,6-isopropylidene derivative **11** and from the ^1H-NMR coupling constants of 5-H/6-H (J = 4.8 Hz). The absolute stereochemistry of gabosine L (**1**) was determined by Helmchen′s method[6] involving the esterification of all three hydroxy groups of **1** with both enantiomers of 2-phenylbutyric acid. A comparison of the ^1H-NMR data of the derivatives **12** and **13** showed a high-field shift for 5-H of Δδ = 0.1 ppm and for 4-H_2 Δδ = 0.2 ppm in the (2*S*) diastereoisomer **13**. The absolute configuration of gabosine L (**1**) is thus (5*R*,6*R*).





11

8: R = H
9: R = (2R)-C(O)-CH(C_6H_5)-CH_2-CH_3
10: R = (2S)-C(O)-CH(C_6H_5)-CH_2-CH_3



12: R = (2R)-C(O)-CH(C_6H_5)-CH_2-CH_3
13: R = (2S)-C(O)-CH(C_6H_5)-CH_2-CH_3

Scheme 2. Derivatives of gabosine L (**11**, **12**, **13**) and gabosine N (**8**, **9**, **10**)

Gabosine N (2)

The molecular formula $C_7H_{10}O_4$ (M^+: 158.0579) was determined by HREI-MS and ESI-MS. In analogy to gabosine L (**1**) **2** exhibits an α,β-unsaturated carbonyl group [IR: 1674 cm^{-1}; ^{13}C NMR: δ_{DMSO} = 199.3 (s, C-1), 131.8 (s, C-2), 145.5 (d, C-3)]. From the ^{13}C-NMR-spectroscopic data the presence of a methyl group (δ = 14.7, q, C-7) and three methine groups linked to oxygen atoms [δ = 76.5 (d), 75.4 (d), 67.7 (d)] were established. Analysis by ^1H, ^1H-^1H-COSY, ^1H-^{13}C shift-correlation, and HMBC-NMR spectra confirmed the constitution of gabosine N (**2**) to be 4,5,6-trihydroxy-2-methyl-2-cyclohexenone.

The relative configuration of **2** was obtained from the ^1H-NMR coupling constants of 4-H, 5-H and 6-H, measured in CD_3OD: δ = 4.55 (m, 4-H), 4.33 (dt, J = 3.5 Hz, J = 2.5 Hz, 5-H), 4.21 (d, J = 2.5 Hz, 6-H), which indicate that no axial/axial coupling is present in **2**. Thus, the stereochemistry of **2** resembles that of gabosine C (**6**).[2] The 4,5-*cis* configuration was independently confirmed by chemical means by the observation that **2** yields the 4,5-*O*-isopropylidene derivative **8** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid.

The absolute configuration of **2** was determined by Helmchen′s method[6] by a selective esterification of the C-6 hydroxy group of **8** with both enantiomers of 2-phenylbutyric acid. The comparison of the data obtained from the ^1H-NMR spectra of both derivatives, the 2-phenylbutyrates **9** and **10**, indicated in the case of **9** a significant down-field shift for the signal of 5-H (Δδ = 0.10 ppm) and for the signal of one of the methyl groups of the isopropylidene moiety (Δδ = 0.05 ppm). This is consistent with an (*R*) configuration of C-6 in **8** and, as a consequence also in **2**. Therefore, gabosine N (**2**) was proven to be (4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-methyl-2-cyclohexenone. The identical absolute stereochemistry of gabosine N (**2**) and C (**6**) is in agreement with the optical rotation value {**2**: $[\alpha]_D^{20}$ = −152 (c = 0.89 in H_2O); **6**: $[\alpha]_D^{20}$ = −168 (c = 1.0 in H_2O)}. Furthermore, the absolute configuration of **2** was investigated by X-ray analysis. The crystal structure (Figure 1) was solved by direct methods using SHELXS-97[7] and refined against F^2 on all data by full-matrix least squares with SHELXL-97.[8] The results are consistent with the data derived from the stereochemical investigations from NMR analysis of the pure metabolites and its derivatives.

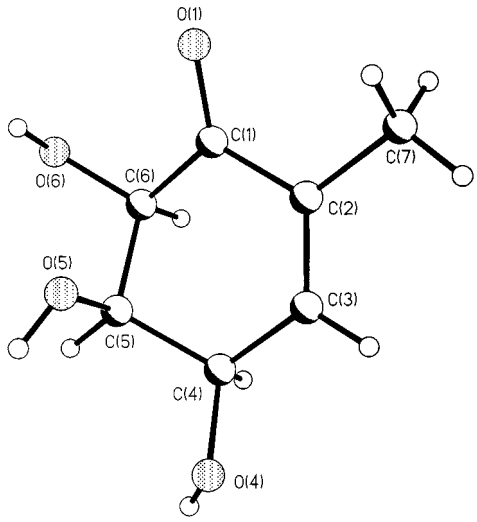


Figure 1. Perspective view of gabosine N (**2**)

Gabosine O (3)

In an analogous manner to **2** the constitution of gabosine O (**3**) was determined as 2,3,4-trihydroxy-6-methyl-cyclohexanone by detailed analysis of the ^1H-, ^{13}C-, ^1H-^{13}C

COSY-NMR spectra in combination with the results from HREI-MS (M^+: 160.0740; $C_7H_{12}O_4$) and ESI-MS. The relative stereochemistry of gabosine O (**3**) resulted from information relating to the four methine proton signals [δ = 4.25 (dd, J = 3.1 Hz, J = 1.3 Hz, 2-H), 4.19 (dd, J = 3.1 Hz, J = 1.8 Hz, 3-H), 4.17 (ddd, J = 10.2 Hz, J = 5.0 Hz, J = 1.8 Hz, 4-H), 2.53 (dddq, J = 13.1 Hz, J = 6.5 Hz, J = 6.0 Hz, J = 1.3 Hz, 6-H), 1.82 (ddd, J = 14.1 Hz, J = 13.1 Hz, J = 10.2 Hz, 5-H_α)]. The coupling constant between 5-H_α and 6-H (13.1 Hz) points to the axial position of both protons, $J_{4,5\alpha}$ = 10.2 Hz indicates the axial position of 4-H, and $J_{3,4}$ = 1.8 Hz is a result of the equatorial position of 3-H. However, the dd signal of 2-H obviously arises from W coupling also suggesting an axial position of this proton. Thus, all functional groups of **3** are located in *syn*-facial positions resulting in the relative stereochemistry depicted in **3**.

DNA-Binding Studies

For the DNA-binding studies a new approach on the basis of the chemical screening detection method making use of TLC analysis was applied.[5] The binding of pure compounds towards DNA can be measured by one-dimensional RP_{18}-HPTLC [solvent system: methanol/ammonium acetate (1 M), 4:1] in which homogenized random DNA was applied together with the test compound at the starting spot of the chromatogram. Analysis is performed via differences in R_f values (R_{f2}/R_{f1}) in comparison to the reference (TLC analysis of the test compounds: without DNA R_{f1}; with DNA R_{f2}). Values of the quotient R_{f2}/R_{f1} smaller than 1 indicates DNA binding. The results from the TLC method were proven independently by DNA melting curves.[5]

Gabosines L (**1**), N (**2**) and O (**3**) as well as several other gabosines and derivatives available were examined. The R_{f2}/R_{f1} values as well as the temperature shifts (ΔT_m) in the melting of DNA (with and without compound) are shown in Table 1. Based on the DNA-binding studies with the TLC method gabosines A (**4**), B (**5**), F (**7**), N (**2**) and O (**3**) as well as the derivatives **8**−**10** showed significant but weak binding properties, while gabosines E, H, and J were found to be non-binders. Although the shifts of the DNA melting points could be observed for the DNA-binding gabosines, the values are low. This might be due to the fact that the low-molecular weight gabosines (< 200 g/mol) are causing only weak destabilizing effects.

Experimental Section

General Methods: Melting points: Büchi melting point B-545 (values not corrected). − ^1H and ^{13}C NMR spectra: Bruker DPX-300 and DPX-500 (XWIN NMR software, Version 1.3) and Varian VXR-200 (Varian standard software). The multiplicity of the ^{13}C-NMR values were assigned by attached proton) test (APT) or distortionless enhancement by polarization transfer (DEPT) techniques. − MS: High-resolution EI mass spectra were recorded with an AMD-402 instrument of *BE* geometry equipped with direct inlet system (AMD intectra Harpstedt, Germany). Electrospray MS were recorded by use of a triple quadrupole mass spectrometer Quattro (VG Biotech, Altrincham, England) and Finnigan MAT 311A (EI: 70 eV, direct inlet, high resolution with perfluorokerosine as a standard). − IR spectra: Perkin−Elmer model 298 (KBr, discs). − UV spectra: Kontron Uvikon 860. − Optical rotation values: Perkin−Elmer 241. − Fermentation: 10-L, 25-L and 50-L fermentor Braun Diessel. − TLC: Silica gel plates (HPTLC ready-to-use plates, silica gel $60F_{254}$ on aluminum foil or glass, Merck). − LC: silica gel 60 (0.040−0.063 mm, Merck). − Sephadex-LH 20 (Pharmacia). − Soybean, mannitol and rolled oats: Difco Ltd.

Fermentation: The strains GT 041230 and GT 051024 were cultivated in 300-mL Erlenmeyer flasks containing 100 mL of medium A (soybean meal 2%, mannitol 2%, pH = 7.2 prior to sterilization) on rotary shakers (180 rpm) for 3 d at 28°C. These cultures were used for metabolite pattern analysis with the chemical screening method[1][2] as well as for inoculation of fermentors (for GT 051024: 50 L × 2 scale; GT 041230: 25 L) containing medium A (180 rpm, aeration: 4 L/min, 28°C, 4 d). − The strain S 1096 was cultivated in 300-mL Erlenmeyer flasks containing 100 mL of medium A on rotary shakers (180 rpm) for 3 d at 30°C. A 10-L fermentor (200 rpm, aeration: 4 L/min, 30°C, 4 d) containing a medium of rolled oats (2%, cooked and filtered) was inoculated with these cultures.

Isolation and Purification of Gabosines: Each of the obtained fermentation broths of GT 041230, GT 051024 and S 1096 was separated by filtration and the culture filtrates were lyophilized. The lyophilisate of S 1096 (54 g) was dissolved in 500 mL of methanol, filtered and dried. Two fractions were obtained by chromatography of the crude product (23 g) on silica gel (9.0 × 26 cm, chloroform/methanol, 7:3). Chromatography of the first fraction on Sephadex LH-20 (3.0 × 80 cm, acetone) and of the second fraction on Sephadex LH-20 (3.0 × 90 cm, methanol) yielded 860 mg of gabosine A (**4**, 86 mg/L), 2.05 g of gabosine B (**5**, 205 mg/L), 490 mg of gabosine C (**6**, 49 mg/L), 250 mg of gabosine L (**1**, 25 mg/L) and 150 mg of gabosine N (**2**, 15 mg/L). The obtained crude product from GT 041230 (30 g) was dissolved in 500 mL of methanol, filtered and dried. The dark brown material obtained (13 g) was chromatographed on a silica gel column (7.0 × 40 cm, petroleum ether/ethyl acetate/methanol, 5:5:1) to yield 100 mg of pure gabosine N (**2**, 4.0 mg/L). The crude product from GT 051024 (40 g) was chro-

Table 1. DNA-binding properties of selected gabosines and derivatives; the R_{f2}/R_{f1} values (TLC method; R_{f1} without DNA; R_{f2} with DNA) as well as the temperature shifts (ΔT_m) in the melting of DNA (with and without test compound) are shown

Gabosine/ derivative	L (**1**)	N (**2**)	O (**3**)	A (**4**)	B (**5**)	C (**6**)	E	F (**7**)	H	J	**8**	**9**	**10**
TLC method: R_{f2}/R_{f1}	0.97	0.89	0.93	0.85	0.85	0.88	1	0.82	1	1	0.65	0.72	0.68
DNA melting point: ΔT_m [°C]	−1.0	1.0	0.2	0.2	0.3	1.0	0	0.4	0.1	0.1	1.0	1.0	1.0

matographed on a silica gel column (7.0 × 40 cm; ethyl acetate/methanol, 12:1) and was further purified by repeated chromatography on a Sephadex LH-20 column (2.5 × 100 cm, methanol) to yield 2 mg of gabosine O (**3**, 0.02 mg/L) and 80 mg of gabosine B (**5**, 0.8 mg/L).

Gabosine L [(5*R*,6*R*)-2,5,6-Trihydroxy-3-methyl-2-cyclohexenone] (1): Light brown oil, $[\alpha]_D^{20} = -13.0$ (c = 0.10, methanol). − IR (KBr): $\tilde{\nu}$ = 2306, 2927, 1679, 1651 cm^{-1}. − UV (methanol): λ_{max} (ε) = 272 nm (6217). − HREI MS: calcd. for $C_7H_{10}O_4$ 158.0579, found 158.0579 [M^+]. − ^{1}H NMR (200 MHz, CD_3OD): δ = 4.21−4.31 (2 H, m, 5-H and 6-H), 2.76 (1 H, qdd, J = 18.1 Hz, J = 3.2 Hz, J = 1.5 Hz, 4-H_{eq}), 2.44 (1 H, dd, J = 18.1 Hz, J = 3.0 Hz, J = 0.8 Hz, 4-H_{ax}), 1.85 (3 H, s, 7-H_3). − ^{13}C NMR (50.3 MHz, CD_3OD): δ = 195.0 (s, C-1), 143.8 (s, C-3), 127.4 (s, C-2), 76.5 (d, C-6), 71.6 (d, C-5), 37.0 (t, C-4), 17.0 (q, C-7).

Gabosine N [(4*R*,5*R*,6*R*)-4,5,6-Trihydroxy-2-methyl-2-cyclohexenone] (2): Colorless needles. M.p. 182.5−183.3 °C. − $[\alpha]_D^{20}$ = −152.0 (c = 0.89, H_2O). − IR (KBr): $\tilde{\nu}$ = 3430, 2935, 1674, 1341 cm^{-1}. − UV (ethanol): λ_{max} (ε) = 230 nm (12500). − UV (ethanol, H^+): λ_{max} (ε) = 230 nm (12500). − UV (ethanol, OH^-): λ_{max} (ε) = 210 nm (14600), 321 (6600). − ESI MS (positive ion); m/z: 158.9 [M^+ + H], 181.0 [M^+ + Na]. − ESI MS (negative ion); m/z: 157.1 [M^- − H], 139.0 [M^- − H_2O − H]. − HREI MS: calcd. for $C_7H_{10}O_4$ 158.0579, found 158.0576 [M^+]; calcd. for $C_7H_8O_3$ 140.0473, found 140.0483 [M^+ − H_2O]. − ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 6.39 (1 H, qd, J = 1.5 Hz, J = 2.5 Hz, 3-H); 5.12 (1 H, d, J = 7.6 Hz, 5-OH), 4.92 (1 H, d, J = 4.9 Hz, 4-OH), 4.90 (1 H, d, J = 3.5 Hz, 6-OH), 4.44 (1 H, m, 5-H), 4.12 (2 H, m, 4-H, 6-H), 1.68 (3 H, dd, J = 2.5 Hz, 1.5 Hz, 7-H_3). − ^{1}H NMR (300 MHz, CD_3OD). δ = 6.48 (1 H, dq, J = 2.5 Hz, J = 1.5 Hz, 3-H), 4.55 (1 H, m, 4-H), 4.33 (1 H, dt, J = 3.5 Hz, J = 2.5 Hz, 5-H), 4.21 (1 H, d, J = 2.5 Hz, 6-H), 1.80 (3 H, d, J = 1.5 Hz, 7-H_3). − ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 199.3 (s, C-1), 145.5 (d, C-3), 131.8 (s, C-2), 76.5 (d, C-4), 75.4 (d, C-6), 67.7 (d, C-5), 14.7 (q, C-7).

X-ray Crystal Analysis of Gabosine N (2): Compound **2** (empirical formula: $C_7H_{10}O_4$, M_r = 158.15) was crystallized from MeOH at 4 °C. Crystal size 0.2 × 0.3 × 0.3 mm, orthorhombic, space group $P2_12_12_1$, a = 462.4(8), b = 1056.7(8), c = 1470.4(2) pm; V = 0.7184(2) nm^3, Z = 4, $D_{calcd.}$ = 1.462 mg/m^3, μ = 0.121 mm^{-1}, Stoe-Siemens-Huber four-circle diffractometer coupled to a Siemens CCD area detector with graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å), −140 °C, Θ range = 2.25−25°, 8474 reflections measured, 1290 unique, structure solved by direct methods using SHELXS-97[7] and refined against F^2 on all data by full-matrix least squares with SHELXL-97.[8] A riding model with idealized hydrogen geometry was employed. The anisotropic refinement converged at R_1 [$F > 4\sigma(F)$] = 0.0496; wR_2 (F^2) = 0.1240. Crystallographic data (excluding structure factors) for gabosine N have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-127418. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Gabosine O [2,3,4-Trihydroxy-6-methylcyclohexanone] (3): Colorless crystals. M.p. 88−89 °C. − $[\alpha]_D^{20}$ = −21.0 (c = 0.1, methanol). − IR (KBr): $\tilde{\nu}$ = 3393, 2965, 2923, 1710, 1076 cm^{-1}. − HREI MS: calcd. for $C_7H_{12}O_4$ 160.0736, found 160.0740 [M^+], 142.0637 [M^+ − H_2O, $C_7H_{10}O_3$]. − ESI MS (positive ion); m/z = 161.1 [M^+ + H], 183.0 [M^+ + Na]. − ^{1}H NMR (300 MHz, CD_3OD): δ = 4.25 (1 H, dd, J = 1.3 Hz, J = 3.1 Hz, 2-H), 4.19 (1 H, dd, J = 3.1 Hz, J = 1.8 Hz, 3-H), 4.17 (1 H, ddd, J = 1.8 Hz, J = 5.0 Hz, J = 10.2 Hz, 4-H), 2.53 (1 H, dddq, J = 13.1 Hz, J = 1.3 Hz, J = 6.0 Hz, J = 6.5 Hz, 6-H), 2.00 (1 H, ddd, J = 5.0 Hz, J = 14.1 Hz, J = 6.0 Hz, 5-H_β), 1.82 (1 H, ddd, J = 13.1 Hz, J = 14.1 Hz, J = 10.2 Hz, 5-H_α), 1.03 (3 H, d, J = 6.5 Hz, 7-H_3). − ^{13}C NMR (300 MHz, CD_3OD): δ = 212.0 (s, C-1), 78.5 (d, C-3), 76.9 (d, C-2), 69.5 (d, C-4), 39.7 (d, C-6), 37.9 (t, C-5), 13.9 (q, C-7).

4,5-*O*-Isopropylidenegabosine N (8): Compound **2** (27 mg) was dissolved in dimethoxypropane (3 mL) containing *p*-toluenesulfonic acid (1 mg). After stirring at room temperature for 14 h, the mixture was neutralized with Na_2CO_3, concentrated in vacuo and the residue obtained was chromatographed immediately on silica gel (column 50 × 1 cm, chloroform/methanol, 50:1) to yield 30 mg (90%) of **8** as a white powder: − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.38 (1 H, qd, J = 1.5 Hz, J = 3.0 Hz, 3-H), 4.88 (1 H, m, 4-H), 4.78 (1 H, m, 5 H), 4.40 (1 H, d, J = 3.2 Hz, 6-H), 3.75 (1 H, 6-OH), 1.89 (3 H, t, J = 1.5 Hz, 7-H_3), 1.39 (3 H, s, 9-H_3), 1.31 (3 H, s, 10-H_3). − ^{13}C NMR (75.0 MHz, $CDCl_3$): δ = 197.4 (s, C-1), 140.8 (d, C-3), 133.2 (s, C-2), 111.3 (s, C-8), 77.6, 72.5 and 72.1 (all d, C-4, C-5 and C-6), 27.7 and 26.9 (both q, C-9, and C-10), 15.2 (q, C-7). − HREI MS: calcd. for $C_{10}H_{14}O_4$ 198.0892, found 198.0895 [M^+]; calcd. for $C_9H_{11}O_4$ 183.0657, found 183.0655 [M^+ − CH_3].

4,5-*O*-Isopropylidene-6-*O*-[(2*R*)-2-phenylbutyryl]gabosine N (9): (2*R*)-2-Phenylbutyric acid (10 mg), dicyclohexylcarbodiimide (12 mg) and 4-(dimethylamino)pyridine (8 mg) were added to a 10-mL flask containing dichloromethane (3 mL). The mixture was stirred and cooled to 0 °C. Then **8** (10.3 mg), dissolved in dichloromethane (2 mL), was added and the mixture was slowly heated to room temperature. After 12 h, water (4 mL) was added and the aqueous phase was extracted three times with 5 mL of chloroform. The combined organic layers were concentrated to dryness and the residue was chromatographed on silica gel (column 50 × 1 cm, chloroform/methanol, 99:1) to yield 5.0 mg (30%) of colorless oily **9**. − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22−7.44 (5 H, m, aromatic H), 6.34 (1 H, qd, J = 1.5 Hz, J = 2.5 Hz, 3-H), 5.69 (1 H, d, J = 3.2 Hz, 6-H), 4.83 (1 H, m, 4-H), 4.72 (1 H, m, 5-H), 3.72 (1 H, t, J = 7.7 Hz, 2′-H), 2.20 (1 H, m, 3′-H_b), 1.90 (1 H, m, 3′-H_a), 1.83 (3 H, t, J = 1.5 Hz, 7-H_3), 1.39 and 1.34 (6 H, both s, 9-H_3 and 10-H_3), 0.98 (3 H, t, J = 7.4 Hz, 4′-H_3). − HREI MS: calcd. for $C_{20}H_{24}O_5$ 344.1624; found 344.1616 [M^+]; calcd. for $C_{19}H_{21}O_5$ 329.1389, found 329.1394 [M^+ − CH_3].

4,5-*O*-Isopropylidene-6-*O*-[(2*S*)-2-phenylbutyryl]gabosine N (10): Using the same synthetic conditions and workup procedure as those for **9**, compound **10** (4.0 mg, 29%) was obtained as a colorless oil from **8** (8 mg) and (2*S*)-2-phenylbutyric acid (9 mg). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22−7.43 (5 H, m, aromatic H), 6.34 (1 H, qd, J = 1.5 Hz, J = 2.5 Hz, 3-H), 5.55 (1 H, d, J = 3.2 Hz, 6-H), 4.80 (1 H, m, 4-H), 4.62 (1 H, m, 5-H), 3.75 (1 H, t, J = 7.6 Hz, 2′-H), 2.28 (1 H, m, 3′-H_b), 1.87 (3 H, t, J = 1.5 Hz, 7-H_3), 1.83 (1 H, m, 3′-H_a), 1.34 (6 H, s, 9-H_3 and 10-H_3), 1.11 (3 H, t, J = 7.4 Hz, 4′-H_3). − HREI MS: calcd. for $C_{20}H_{24}O_5$ 344.1624, found 344.1614 [M^+].

5,6-*O*-Isopropylidenegabosine L (11): 1 (10 mg) was dissolved in acetone (1 mL) containing iodine (4 mg). After stirring at room temperature for 4 h, the remaining iodine was reduced with $Na_2S_2O_3$ solution. The mixture was extracted with $CHCl_3$ and the extract was concentrated in vacuum to yield pure **11** (11 mg, 100%) as a white powder. − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.54 (1 H, ddd, J = 4.8 Hz, J = 4.8 Hz, J = 1.9 Hz, 5-H), 4.39 (1 H, d, J = 4.8 Hz, 6-H), 2.82 (1 H, qdd, J = 19.3 Hz, 4.8 Hz, 1.8 Hz, 4-H_{eq}), 2.74 (1 H, dm, J = 19.3 Hz, 4-H_{ax}), 1.93 (3 H, s, 7-H_3), 1.38 and 1.30 (6 H, both s, 9-H_3 and 10-H_3). − ^{13}C NMR (125.7 MHz,

$CDCl_3$): δ = 191.0 (s, C-1), 142.4 (s, C-3), 127.5 (s, C-2), 109.4 (s, C-8), 74.7 (d, C-6), 72.3 (d, C-5), 30.7 (t, C-4), 27.6 and 26.0 (both q, C-9 and C-10), 17.2 (q, C-7).

2,5,6-Tri-*O*-[(2*R*)-2-phenylbutyryl]gabosine L (12): Using the same synthetic conditions and workup procedure as those for **9**, colorless oily **12** (10 mg, 66%) was obtained from **1** (5.0 mg) and (2R)-2-phenylbutyric acid (35 mg). − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.14−7.35 (15 H, m, aromatic H), 5.54 (1 H, d, *J* = 3.0 Hz, 6-H), 5.43 (1 H, ddd, *J* = 3.0 Hz, *J* = 3.0 Hz, *J* = 3.0 Hz, 5-H), 3.69, 3.40 and 3.69 (3 H, all t, *J* = 7.3 Hz, 3 × 2′-H), 2.82 (1 H, dm_c, *J* = 18.8 Hz, 4-H_{eq}), 2.46 (1 H, dd, *J* = 18.8 Hz, *J* = 2.9 Hz, 4-H_{ax}), 2.15−2.24 and 1.59−2.05 (6 H, m, 3 × 3′-H_2), 1.40 (3 H, s, 7-H_3), 0.94, 0.85 and 0.78 (9 H, all t, *J* = 7.3 Hz, 3 × 4′-H_3). − DCI MS; *m*/*z*: 614.3 [$M^+ + NH_3 + H$].

2,5,6-Tri-*O*-[(2*S*)-2-phenylbutyryl]gabosine L (13): Using the same conditions and workup procedure as those for **10**, colorless oily **13** (7 mg, 25%) was obtained from **1** (6.0 mg) and (2R)-2-phenylbutyric acid (35 mg). − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.21−7.39 (15 H, m, aromatic H), 5.55 (1 H, d, *J* = 3.0 Hz, 6-H), 5.30 (1 H, ddd, *J* = 3.0 Hz, *J* = 3.0 Hz, *J* = 3.0 Hz, 5-H), 3.68, 3.49 and 3.37 (3 H, all t, *J* = 7.3 Hz, 3 × 2′-H), 2.68 (1 H, dm, *J* = 18.5 Hz, 4-H_{eq}), 2.22−2.33 and 1.69−2.03 (7 H, m, 3 × 3′-H_2 and 4-H_{ax}), 1.31 (3 H, s, 7-H_3), 1.01, 0.91 and 0.83 (9 H, all t, *J* = 7.3 Hz, 3 × 4′-H_3). − DCI MS; *m*/*z* = 614.3 [$M^+ + NH_3 + H$], 596.3 [M^+].

DNA-Binding Studies: Pure compounds (5 μg, dissolved in methanol, 1 mg/mL) were spotted onto silica gel plates (Merck, RP-18 WF_{254S} HPTLC plates on glass) with and without additional homogenized salmon sperm DNA (4 μg, dissolved in water, 1 mg/mL).[5] The plates were chromatographed with methanol/ammonium acetate (1 M in water) as solvent system and spots located using anisaldehyde/H_2SO_4.

Measurement of DNA-Melting Curves: Melting curves were measured using a Cary 1E UV/Vis spectrophotometer (Varian) equipped with a thermo-adjustable cuvette holder. Temperature gradients were 1°C/min (20−98°C). Melting temperatures (T_m) were monitored at 260 nm and resulted in the determination of melting temperature shifts (ΔT_m) of DNA (with and without compound). Samples for DNA−ligand interaction studies contained 25 μg/mL homogenized salmon sperm DNA dissolved in 50 mM NaCl.

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