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Tjipanazoles, New Antifungal Agents from the Blue-Green Alga *Tolypothrix tjipanasensis*

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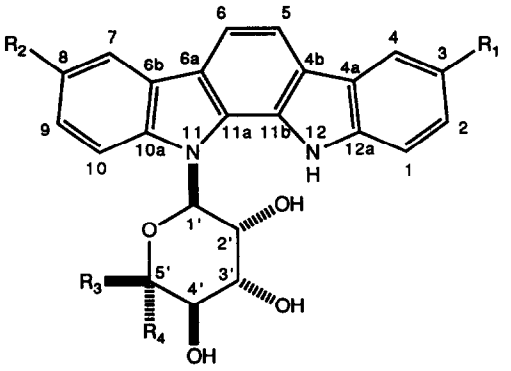
Abstract Bioactivity-directed isolation of the extract of the cyanophyte *Tolypothrix tjipanasensis* has led to the isolation of fifteen new N-glycosides of indolo[2,3-a]carbazoles designated tjipanazoles A1, A2, B, C1, C2, C3, C4, D, E, F1, F2, G1, G2, I and J The structures of the alkaloids were determined by physical methods, chemical degradation and synthesis Tjipanazole J is the only compound having the pyrrolo[3,4-c] ring of previously described indolo[2,3-a]carbazoles

In screening extracts of blue-green algae for antifungal activity, we have found that indolo[2,3-a]carbazoles, which we have named tjipanazoles, are responsible for the moderate fungicidal activity of the lipophilic extract of *Tolypothrix tjipanasensis* (strain DB-1-1) against *Candida albicans*, *Trichophyton mentagrophytes*, and *Aspergillus flavus* [1] Unlike indolo[2,3-a]carbazoles from actinomycetes and slime molds,[2-7] fourteen of the fifteen tjipanazoles described in this paper lack the pyrrolo[3,4-c] ring of analogs such as rebeccamycin[2] and K252-d [7]

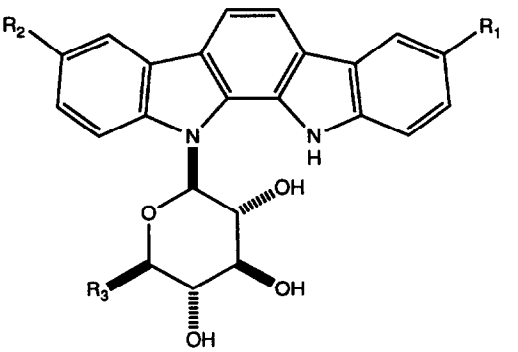
Isolation and Structure Determination

Tolypothrix tjipanasensis De Wild, isolated from a soil sample collected in Vero Beach, Florida, was mass cultured in a liquid medium (modified A_3M_7) [8] After 2-3 weeks the alga was harvested by filtration, lyophilized and extracted with 70% ethanol Successive gel and reverse-phase chromatography of the extract followed by normal or reverse phase HPLC resulted in the isolation of fifteen pure tjipanazoles NMR and HPLC analyses were used to monitor the purification of each alkaloid

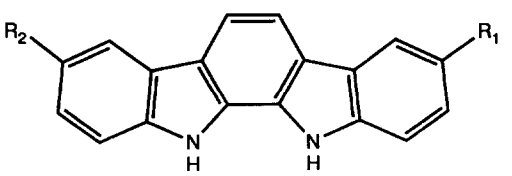
A 4 1 mixture of tjipanazoles A1 and A2, obtained in 1 25% yield from the dried alga after successive gel and reverse-phase chromatography, could be separated into pure A1 and A2 by repeated normal phase HPLC on silica The FD mass spectra of both A1 and A2 exhibited intense 10 7 1 bis-chlorine-containing molecular ion clusters at m/z 470, 472 and 474 and their UV spectra were characteristic of indolo[2,3-a]carbazoles The proton NMR spectral data for tjipanazole A1 (Table 1), the major alkaloid, indicated the presence of one 1,2,3,4-tetrasubstituted and two 1,2,4-trisubstituted benzenoid rings, an indole-type NH group, and a hexopyranose ring where the anomeric carbon was attached to a nitrogen in the aromatic portion of the molecule The ^{13}C NMR (Table 2) and mass spectral data were consistent with the molecular formula



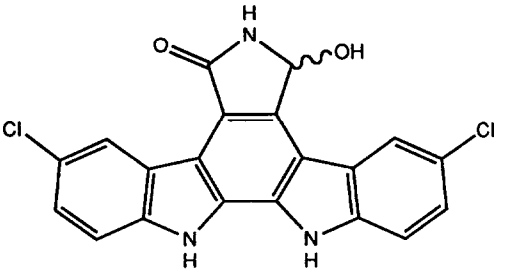
tjipanazole	R_1	R_2	R_3	R_4
A1	Cl	Cl	Me	H
A2	Cl	Cl	H	Me
C1	Cl	H	Me	H
C2	H	Cl	Me	H
C3	Cl	H	H	Me
C4	H	Cl	H	Me
G1	H	H	Me	H
G2	H	H	H	Me



tjipanazole	R_1	R_2	R_3
B	Cl	Cl	H
E	Cl	Cl	CH_2OH
F1	Cl	H	H
F2	H	Cl	H



tjipanazole	R_1	R_2
D	Cl	Cl
I	Cl	H



tjipanazole J

$C_{24}H_{20}N_2O_4Cl_2$ Proton-proton decoupling and NOE experiments suggested that the sugar moiety was a β-6-deoxygulosyl unit which was attached to one of the nitrogens of a 3,8-dichloroindolo[2,3-a]carbazole (Fig 1) In tjipanazole A2 the sugar moiety appeared to be a β-rhamnosyl unit from comparison of the proton and carbon chemical shifts (Tables 1 and 2) with values reported for K-252d [7]

The structures of tjipanazoles A1 and A2 were confirmed by acid hydrolysis to the aglycone 3,8-dichloroindolo[2,3-a]carbazole and the free sugars 6-deoxy-D-gulose and L-rhamnose, respectively The aglycone was identical in all respects with tjipanazole D and a synthetic sample produced by coupling, in the presence of air, two equivalents of p-chlorophenylhydrazine with 1,2-cyclohexanedione in a Fischer indole type synthesis (Scheme 1) To determine the absolute stereochemistries of the sugars, the methyl glycoside tribenzoate esters of the sugars were prepared and the β-methylglucosides were differentiated from the α-anomers by proton NMR analysis The CD spectrum of the β derivative of 6-deoxygulose showed a negative Cotton effect, strongly suggesting that this sugar was D [9] The CD was attributed mainly to the interaction of the equatorial benzoate on C-2 and the axial benzoate on C-3 (strong negative Cotton effect) and not to the interaction of the equatorial benzoate on C-2 with the axial benzoate on C-4 (weak positive Cotton effect) or to the interaction of the axial benzoates on C-3 and C-4 (no Cotton effect) The CD spectrum of the α derivative of rhamnose showed a positive Cotton effect, identical with that observed in the CD spectrum of an authentic sample derived from L-rhamnose

Tjipanazole B was obtained in 0 25% yield from the dried alga Its proton NMR spectrum at 25 °C was complex due to the presence of a 2 1 mixture of two conformational isomers At 100 °C, however, the spectrum resolved into a single set of signals which indicated the presence of a 3,8-dichloroindolo[2,3-a]carbazole bearing a pentopyranose The ^1H and ^{13}C NMR (at 100 °C, see Table 2) and mass spectral data were consistent with the molecular formula $C_{23}H_{18}N_2O_4Cl_2$ Proton-proton decoupling experiments suggested that the sugar moiety was a β-xylosyl unit The structure of tjipanazole B was confirmed by acid hydrolysis to tjipanazole D and D-xylose The absolute stereochemistry of the xylose was deduced by converting the sugar to a mixture of α and β-methyl-D-xyloside triacetates, separating the isomers by chromatography, and comparing the optical rotation of the α-anomer with the value reported in the literature [10,11]

Tjipanazole E was isolated as a minor component The ^1H NMR data (Table 1 and Experimental Section) suggested that a β-glucosyl unit was attached to the 3,8-dichloroindolol[2 3-a]carbazole and the ^{13}C NMR (Table 2) and mass spectral data supported the implied molecular formula $C_{24}H_{20}N_2O_5Cl_2$ The structure was confirmed by acid hydrolysis to tjipanazole D and D-glucose and the absolute stereochemistry of the sugar was rigorously established by synthesis of tjipanazole E, viz N-glycosidation of tjipanazole D using a procedure described by Robins and coworkers [12]

Minor amounts of two N-glycosides of indolo[2,3-a]carbazole were also found, although indolo[2,3-a]carbazole itself was not present in sufficient amount to be detected Tjipanazole G1 was determined to be N-β-6-deoxygulosylindolo[2,3-a]carbazole by spectral analysis and tjipanazole G2 was shown to be N-β-rhamnosylindolo[2,3-a]carbazole by synthesis

Tjipanazole I, another minor component, was shown to be 3-chloroindolol[2,3-a]carbazole by straightforward UV, NMR and mass spectral analyses Several other minor tjipanazoles in the alga were found

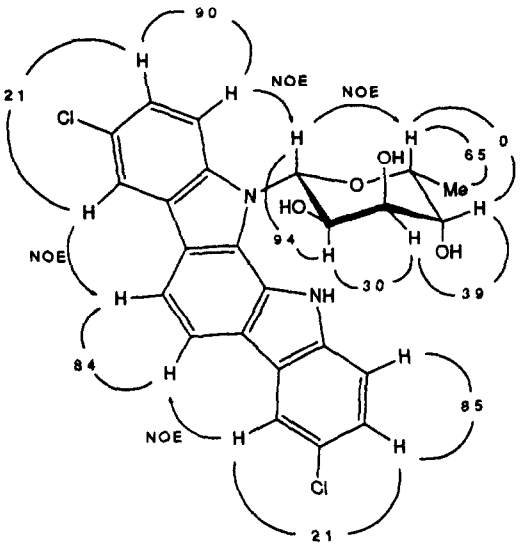
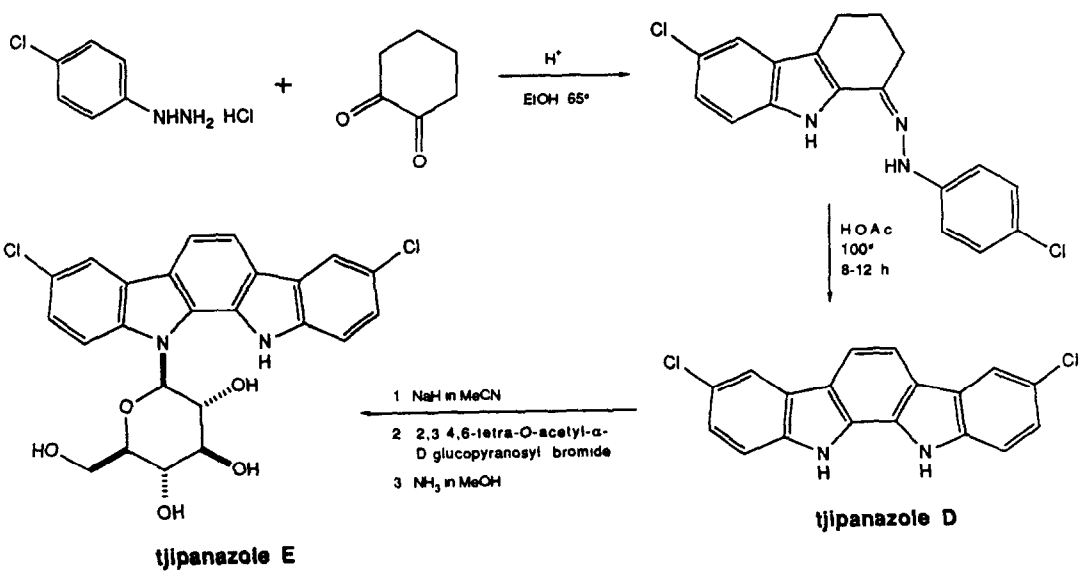


Figure 1 ^{1}H-^{1}H coupling constants and NOEs for tjipanazole A1



tjipanazole E

tjipanazole D

Scheme 1

to be N-β-glycosides of tjipanazole I, e g a β-6-deoxygulosyl unit was attached to N-11 and N-12 of 3-chloroindolo[2,3-a]carbazole in tjipanazoles C1 and C-2, whereas a β-rhamnosyl unit was attached to these nitrogens in tjipanazoles C3 and C4 and a β-xylosyl unit to these nitrogens in tjipanazoles F1 and F2, respectively Proton-proton decoupling and NOE experiments, similar to those shown in Fig 1, established the nature of each sugar unit and its regiochemistry with respect to the chloro substituent.

Tjipanazole J was isolated from the fraction containing D and I Its UV spectrum suggested similarity to UCN-01[4] and the FAB mass spectrum was consistent with the molecular formula $C_{20}H_{11}N_3O_2Cl_2$ The IR spectrum revealed the presence of a lactam ring Examination of the proton NMR spectrum showed that J was a bis-chlorinated indolocarbazole which contained the γ-hydroxy-γ-lactam found in UCN-01 Tjipanazole J was the only compound possessing the pyrrolol[3,4-c] ring of previously described indolo[2,3-a]carbazoles

Biological Results

The tjipanazoles failed to offer any significant in vivo protection in mice systemically infected with *Candida albicans* In tests against several phytopathogenic fungi, however, tjipanazoles A1 and A2 exhibited appreciable selective fungicidal activity against rice blast and leaf rust wheat infections[13]

The tjipanazoles showed only weak, non-selective cytotoxicity against leukemia and solid tumor cell lines in the Corbett assay[14] and were inactive against tumor cells implanted in mice This is in contrast to indolo[2,3-a]carbazoles possessing a pyrrolo[3,4-c] ring Rebeccamycin, for example, which exhibits significant activity against P-388 and L1210 leukemia and B-16 melanoma in mice and inhibits the growth of human lung adenocarcinoma cells in vitro,[2] has been reported to produce single-strand breaks in DNA and is currently being considered for clinical evaluation in the treatment of human cancer Another compound, staurosporine, which is a potent protein kinase inhibitor,[6] shows activity against several experimental tumors in vitro

Finally, the tjipanazoles were found to be inactive as inhibitors of rat brain protein kinase C at concentrations up to 10^{-6} M, which is quite high compared to concentrations in the range of 10-100 X 10^{-9} M normally seen for indolocarbazoles such as UCN-01 and K-252d[4,7]

Experimental Section

General Procedures NMR spectra were determined at 500 and/or 300 MHz for ^1H and 125 and/or 75 MHz for ^{13}C Proton chemical shifts are referenced in DMSO-d_6 to the residual DMSO-d_5 signal (2 52 ppm) and in acetone-d_6 to the residual acetone-d_5 signal (2 04 ppm) Carbon-13 chemical shifts are referenced in DMSO-d_6 and acetone-d_6 to the solvent (39 5 and 29 8 ppm, respectively) Qualitative homonuclear ^1H NOEs were obtained in DMSO-d_6 by selective irradiation for 2 s using 30-32 dB of gated decoupler power (hetero mode), followed by data acquisition (decoupler off) with no recycle delay, subtraction of this on-resonance FID from an off-resonance FID resulted in a difference FID which after processing gave an NOE difference spectrum Homonuclear ^1H and heteronuclear ^1H-^{13}C connectivities were determined by using phase-cycled 16 step COSY and CSCM (or HMQC) experiments, respectively

Preparative TLC was run on 20 x 20 cm (0 5 mm thickness) EM silica gel F-254 plates in the solvents indicated

Culture Conditions A clonal isolate, designated UH strain DB-1-1 and identified as *Tolypothrix tjipanasensis* De Wild (Scytonemataceae, Nostocales, Cyanophyceae, Cyanophyta) according to the system of Fritsch, was obtained from a soil sample collected at Vero Beach, Florida in the fall of 1985 Repeated subculture on a solidified medium was used to purify the alga Mass cultivation of DB-1-1 was carried out in liquid medium using the procedure previously described for *Hapalosiphon fontinalis* [8]

Isolation of the Tjipanazoles Freeze-dried alga (590 g) was extracted with 45 L of 70 30 EtOH/water with stirring overnight in a refrigerator The filtered extract was concentrated under reduced pressure to a volume of 8 L The concentrate was extracted with ethyl acetate (2 X 2 5 L) using salt to dissociate the emulsion into two phases The combined EtOAc layers were evaporated to give a solid (35 g) which was dissolved in 100 mL of methanol The resulting solution was applied to a column (7 cm x 33 cm) of Sephadex LH-20 (Pharmacia) equilibrated in MeOH and fractions were collected by eluting the column with methanol The fraction that contained tjipanazoles A-C and E-G by TLC and HPLC were combined and evaporated to give 12 2 g of Residue I The slower-moving fractions that contained tjipanazoles D, I, and J resulted in 0 29 g of Residue II

Aliquots (2 5-5 mL) of a solution of Residue I in 45 mL of MeOH were injected onto a 5 cm x 35 cm steel column packed with Matrex C-8 (Amicon) Using 73 27 MeOH/pH 4 8 acetate buffer (0 2% acetic acid adjusted to pH 4 8 with NaOH) at a flow rate of 25 mL/min, 150-175 mL fractions were collected Under these isocratic conditions a partially resolved mixture of tjipanazoles G1/G2, F1/F2, E, C1/C2/C3/C4, and B was eluted from the column As soon as tjipanazole A1/A2 began to co-elute with tjipanazole B, the solvent system was changed to 75 25 MeOH/pH 4 8 acetate buffer and a linear gradient to 9 1 MeOH/pH 4 8 acetate buffer was applied to the column over 25 min to remove the remaining tjipanazoles A and B Fractions were combined into pools on the basis of analytical HPLC Each pool was then concentrated and the aqueous concentrate was extracted with EtOAc The EtOAc extracts were finally evaporated to give Residue III (0 77 g) containing tjipanazoles C1/C2/C3/C4, E, F1/F2 and G1/G2, Residue IV (2 92 g) containing tjipanazoles A1/A2 and B, and Residue V (7 36 g) containing only tjipanazole A1/A2

Separation of tjipanazole A1/A2 into two components A1 and A2 was achieved by preparative HPLC A solution of 76 mg of Residue V in 0 25 mL of THF was injected onto a 2 54 cm x 30 cm column of Chromegabond C-18 (ES Industries) equilibrated in 1 1 THF/pH 4 8 acetate buffer Using a flow rate of 5 mL/min, 2 5-5 mL fractions were collected and analyzed by HPLC (Table 3) The fractions that contained tjipanazole A1 in >95% were combined and concentrated to about 10 mL, the concentrate extracted with EtOAc (2 x 10 mL), and the combined EtOAc extract washed with water and evaporated to afford 55 mg of **tjipanazole A1** The 2 3 mixture of tjipanazoles A1 and A2 in the remaining fractions was recovered and rechromatographed as described above to give 10 mg of **tjipanazole A2** in >98% purity, along with additional tjipanazole A1

To obtain tjipanazole B a 30 mg sample of Residue IV in 0 5 mL of dichloromethane was applied to a preparative 20 x 20 cm TLC plate of silica F-254 (Merck) and developed with 9 1 CH_2Cl_2/MeOH The UV-active band with an R_f of 0 36 was removed from the plate and extracted with CH_2Cl_2/MeOH to give 10 mg of **tjipanazole B**

Residue III (0 77 g) was dissolved in 1 5 mL of MeOH and a 0 5 mL aliquot was subjected to preparative HPLC on a 2 54 x 25 cm column of Zorbax C-18 (DuPont) equilibrated in 78 22 MeOH/pH 4 8 acetate buffer Using the same solvent system at a flow rate of 5 mL/min, 5 mL fractions were collected The following pools were generated on the basis of an analytical HPLC profile and concentrated The concentrates were extracted with EtOAc and the extracts evaporated to give Residue VI (0 31 g) containing tjipanazole C1/C2/C3/C4, Residue VII (0 08 g) containing tjipanazoles E and F1/F2, and Residue VIII (0 03 g) containing tjipanazole G1/G2

Residue VI (0 125 g) in 0 5 mL of dichloromethane was subjected to medium pressure chromatography on a 3 5 x

Table 1. ^{1}H NMR chemical shifts for tjipanazoles

Proton	A_1	A_2	B at 25°		B at 100°	E
			major conformer	minor conformer		
1	7 60 (d)	7 56	7 85 (br)		7 74	7 41
2	7 48 (dd)	7 48	7 36-7 47 (v br)		7 42	7 26
4	8 33 (d)	8 33	8 29 (d)		8 23	8 00
5	8 05 (d)	8 07	7 97-8 13 (v br)		8 04	7 89
6	8 11 (d)	8 07	7 97 8 13 (v br)		7 99	7 80
7	8 33 (d)	8 33	8 29 (d)		8 23	8 01
9	7 48 (dd)	7 48	7 43 (dd)		7 39	7 34
10	7 74 (d)	7 66	7 66 (br d)	7 80	7 81	7 46
12NH	11 72 (br s)	11 55	11 43 (br s)	10 02	10 58	
1'	6 18 (d)	6 31	6 11 (br d)	5 94	6 02 (d)	5 70 (d)
2'	4 38 (ddd)	4 38	3 66 or 4 00 (br)		3 87 (m)	3 69 (dd)
2'OH	5 07 (br d)	5 15	5 17 (br)	4 89	4 80 (br)	
3'	4 16 (d)	4 16	3 66 (br)		3 64 (t)	3 60 (t)
3'OH	5 48 (br d)	5 46	5 29 (br s)		4 80 (br)	
4'	3 89 (t)	4 08	3 66 or 4 00 (br)		3 87 (m)	4 08 (t)
4'OH	6 91 (br d)	6 77	5 13 (br)		4 80 (br)	
5'	4 58 (q)	4 48	3 74 (br)		3 77 (t)	3 67 (dd)
			4 00 (br)	4 44	4 23 (br)	
6'	1 34 (d)	1 70				4 07 (d)
						3 84 (dd)

Table 2 ^{13}C NMR chemical shifts for tjipanazoles

Carbon	A1	A2	B at 25°		B at 100°	E
			major conformer	minor conformer		
1	112 22 (d)	112 22	112 9	112 9	112 8	111 39
2	124 78 (d)	124 78	124 9		124 1	124 94
3	123 30 (s)	123 30	123 5		123 3	124 53
4	119 50 (d)	119 50	119 2	119 3	118 4	119 17
4a	124 27 (s)	124 27	124 4		124 3	125 19
4b	120 60 (s)	120 60	121 6	121 6	121 0	121 79
5	113 37 (d)	113 37	112 9	112 9	112 6	113 66
6	112 06 (d)	112 06	112 3	113 6	111 7	111 58
6a	121 04 (s)	121 04	119 9		120 4	122 19
6b	124 38 (s)	124 38	126 4		125 2	125 11
7	119 14 (d)	119 14	119 2	119 3	118 4	119 21
8	123 90 (s)	123 90	123 8		123 9	125 33
9	124 62 (d)	124 62	124 3	124 6	123 8	125 00
10	111 99 (d)	111 99	115 4	114 4	113 6	112 43
10a	138 40 (s)	138 40	137 7	136 0	137 1	138 77
11a	125 10 (s)	125 10	127 6	125 3	126 2	125 27
11b	126 20 (s)	126 20	125 3		125 6	127 14
12a	137 26 (s)	137 26	137 7	139 2	137 3	138 21
1'	82 38 (d)	77 52	87 4	85 7	86 4	84 98
2'	67 29 (d)	67 22	70 9	73 1	71 7	78 79
3'	71 03 (d)	71 68	77 0	77 8	77 1	76 68
4'	71 83 (d)	71 55	69 5	69 3	69 1	67 40
5	71 08 (d)	76 45 (d)	68 3 (t)	69 2 (t)	68 3 (t)	73 41 (d)
6'	16 57 (q)	15 46 (q)				59 10 (t)

Assignments based on a combination of INADEQUATE, HMQC, and HMBC experiments

35 cm Lobar type C silica column (Merck) using 30 35 35 THF (stabilized)/chloroform/isooctane at a flow rate of 8 mL/min (40-50 psi) Analysis of the various fractions that were collected using analytical HPLC (Zorbax silica column, 4 6 mm x 25 cm, 40 30 30 THF/chloroform/isooctane, 1 5 mL/min, detection at 261 nm) permitted the following pools to be generated Pool I (44 mg, t_R = 4 31 min) which contained tjipanazoles C3/C4 and Pool II (167 mg, t_R = 4 9 min) which contained tjipanazoles C1/C2 The residue in each pool was triturated with 7 mL of hexane The insoluble portion was dissolved in THF and chromatographed on a preparative Zorbax Phenyl HPLC column (2 54 x 25 cm) using 45 55 THF (stabilized)/pH 4 8 acetate buffer at a flow rate of 5 mL/min Fractions were collected and combined on the basis of HPLC analysis (same column and eluant, flow rate of 1 5 mL/min) to give **tjipanazole C1** (2 5 mg, t_R = 12 1 min) and **tjipanazole C2** (12 mg, t_R = 13 0 min) from Pool 2 and **tjipanazole C3** (2 3 mg, t_R = 13 6 min) and **tjipanazole C4** (2 2 mg, t_R = 14 0 min) from Pool 1

Residue VII was dissolved in 2 mL of 9 1 dichloromethane/methanol and subjected to preparative TLC on 20 x 20 cm plates of Merck Silica F254 (0 5 mm thick) in 1 mL portions The plates were developed with 88 12 CH_2Cl_2/MeOH Two major UV-active zones were observed The one with R_f 0 42 consisted of a mixture which nmr analysis showed to be a 5 1 mixture of tjipanazoles F1 and F2 (35 mg) **Tjipanazole F1** (t_R = 6 2 min) and **tjipanazole F2** (t_R = 6 35 min) could be separated on an analytical HPLC column (Zorbax silica column, 4 6 mm x 25 cm, 45 27 5 27 5 THF/chloroform/isooctane, 1 5 mL/min, detection at 261 nm) The zone with R_f 0 29 contained tjipanazole E along with a red pigment Pure **tjipanazole E** (10 mg) was obtained by two further silica TLC separations, first with 70 30 8 CH_2Cl_2/THF/MeOH and then with 88 12 CH_2Cl_2/MeOH

Residue VIII (30 mg) in 0 5 mL of 9 1 CH_2Cl_2/MeOH was further purified by preparative silica TLC, as described above, using 88 12 CH_2Cl_2 The UV-active zone with R_f 0 5 gave 6 mg of a 5 1 mixture of **tjipanazole G1** (t_R = 3 8 min) and **tjipanazole G2** (t_R = 4 1 min) which which could be separated on an analytical HPLC column (Zorbax silica column, 4 6 mm x 25 cm, 45 27 5 27 5 THF/chloroform/isooctane, 1 5 mL/min, detection at 261 nm)

Residue II (0 29 g) was treated with 15 mL of methanol The insoluble portion was dissolved in 100 mL of hot chloroform which, upon cooling, led to tjipanazole D having 80-85% purity This hot chloroform precipitation procedure was repeated to afford pure **tjipanazole D** (150 mg) The methanol-soluble portion of Residue II from above was applied in 1 mL portions to a 2 2 x 32 cm column of Zorbax C-18 (12-17m) equilibrated in 4 1 MeOH/water Using a 5 mL/min flow rate, 20 mL fractions were collected Fractions were pooled on the basis of HPLC analysis to give an additional 20 mg of tjipanazole D, 8 mg of impure tjipanazole I, and 19 mg of impure tjipanazole J Pure **tjipanazole I** (1 5 mg) was obtained by preparative TLC on silica with 4 1 toluene/ethanol (R_f 0 69) Rechromatography on Zorbax C-18 with 9 1 MeOH/water gave 12 mg of **tjipanazole J**

Tjipanazole A1 $[\alpha]_D$ +9 1° ($CHCl_3$, c 1 0), UV (MeOH) λ_{max} nm (ε) 261 (60,950), 294 (23,200), 333 (31,260), 354 (7,340), 371 (4,800), FDMS m/z (rel intensity) 470 (100), 472 (70), 473 (20), 475 (20), high resolution FABMS m/z 470 0807 ($C_{24}H_{20}N_2O_4Cl_2$, 470 0801) ^{1}H NMR (DMSO-d_6) see Table 1 for chemical shift data, coupling constants $J_{1\,2}$ = 8 5 Hz, $J_{2\,4}$ = 2 1, $J_{5\,6}$ = 8 4, $J_{7\,9}$ = 2 1, $J_{9\,10}$ = 9 0, $J_{1'\,2'}$ = 9 4, $J_{2'\,OH}$ = 6 9, $J_{2'\,3'}$ = 3 0, $J_{3'\,OH}$ = 3 7, $J_{3'\,4'}$ = 3 9, $J_{4'\,OH}$ = 3 8, $J_{4'\,5'}$ = 0, $J_{5'\,Me}$ = 6 5 ^{13}C NMR (DMSO-d_6) see Table 2

Tjipanazole A2 $[\alpha]_D$ +25 12° ($CHCl_3$, c 1 0), UV (MeOH) same as A1, high resolution FABMS m/z 470 0807 (calcd for $C_{24}H_{20}N_2O_4Cl_2$, 470 0801) ^{1}H NMR (DMSO-d_6) see Table 1 for chemical shift data, coupling constants $J_{1\,2}$ = 8 6 Hz, $J_{2\,4}$ = 2 1, $J_{5\,6}$ = 8 4, $J_{7\,9}$ = 2 1, $J_{9\,10}$ = 9 0, $J_{1'\,2'}$ = 9 4, $J_{2'\,OH}$ = 7 1, $J_{2'\,3'}$ = 3 0, $J_{3'\,OH}$ = 3 8, $J_{3'\,4'}$ = 3 2, $J_{4'\,OH}$ = 2 4, $J_{4'\,5'}$ = 0, $J_{5'\,Me}$ = 7 3 ^{13}C NMR (DMSO-d_6) see Table 2

Tjipanazole B $[\alpha]_D$ -4 9° ($CHCl_3$, c 1 03), $[\alpha]_D$ +10 5° (1 1 $CHCl_3$/MeOH, c 0 95), UV (MeOH) λ_{max} nm (ε) 259 (59,400), 292 (24,800), 330 (30,400), 349 (8,120), 366 (4,850), FDMS m/z (rel intensity) 456 (100), 458 (50), 460 (25), 462 (10), high resolution FABMS m/z 456 0644 (calcd for $C_{23}H_{18}N_2O_4Cl_2$, 456 0644) ^{1}H NMR

(DMSO-d_6) see Table 1 for chemical shift data at 25 and 100 °C, coupling constants at 100 °C $J_{1,2}$ = 8 4 Hz, $J_{2,4}$ = 2 1, $J_{5,6}$ = 8 3, $J_{7,9}$ = 2 1, $J_{9,10}$ = 8 8, $J_{1',2}$ = 8 9, $J_{2,3}$ = 8 7, $J_{3,4}$ = 8 7, $J_{4,5'}$ = 5 and 10 0, $J_{5,5}$ = -10 0, $J_{5,Me}$ = 7 3 13C NMR (DMSO-d6) see Table 2

Tjipanazole C compounds $[\alpha]_D$ +18 1° ($CHCl_3$, c 1 1), UV (MeOH) λ_{max} nm (ε) 258 (46,200), 271 (43,300), 291 (21,200), 328 (25,700), 349 (6,810), 366 (4,690), FDMS m/z (rel intensity) 436 (100), 438 (20), high resolution FABMS m/z 436 1209 (calcd for $C_{24}H_{21}N_2O_4Cl$, 436 1190) **Tjipanazole C1** ^{1}H NMR (DMSO-d_6) δ 7 53 (d, H1), 7 34 (dd, H2), 8 16 (d, H4), 7 98 (d, H5), 8 05 (d, H6), 8 16 (dd, H7), 7 24 (dd, H8), 7 42 (dd, H9), 7 69 (d, H10), 11 41 (s, 12NH), 6 30 (d, H1'), 4 60 (dd, H2'), 4 36 (dd, H3'), 4 09 (d, H4'), 4 65 (q, H5'), 1 41 (d, H3-6') **Tjipanazole C2** ^{1}H NMR (DMSO-d_6) δ 7 56 (d, H1), 7 36 (dd, H2), 7 19 (dd, H3), 8 18 (dd, H4), 7 98 (d, H5), 8 05 (d, H6), 8 18 (d, H7), 7 40 (dd, H9), 7 70 (d, H10), 11 31 (s, 12NH), 6 28 (d, H1'), 4 62 (dd, H2'), 4 36 (dd, H3'), 4 09 (d, H4'), 4 65 (q, H5'), 1 42 (d, H3-6') **Tjipanazole C3** ^{1}H NMR (DMSO-d_6) δ 7 49 (d, H1), 7 33 (d, H2), 8 17 (dd, H4), 7 99 (d, H5), 8 05 (d, H6), 8 17 (d, H7), 7 24 (dd, H8), 7 43 (dd, H9), 7 69 (d, H10), 11 25 (s, 12NH), 6 48 (d, H1'), 4 69 (dd, H2'), 4 42 (dd, H3'), 4 28 (d, H4'), 4 58 (q, H5'), 1 76 (d, H3-6') **Tjipanazole C4** ^{1}H NMR (DMSO-d_6) δ 7 51 (d, H1), 7 36 (dd, H2), 7 19 (dd, H3), 8 20 (dd, H4), 7 99 (d, H5), 8 04 (d, H6), 8 20 (d, H7), 7 40 (dd, H9), 7 70 (d, H10), 11 16 (s, 12NH), 6 44 (d, H1'), 4 70 (dd, H2'), 4 42 (dd, H3'), 4 27 (d, H4'), 4 59 (q, H5'), 1 76 (d, H3-6')

Tjipanazole D UV (MeOH) λ_{max} nm (ε) 259 (63,100), 291 (25,500), 331 (31,000), 366 (4,030), FDMS m/z (rel intensity) 324 (100), 326 (50), high resolution FABMS m/z 325 0267 (MH^+, calcd for $C_{18}H_{11}N_2Cl_2$, 325 0300) ^{1}H NMR (acetone-d_6) δ 7 60 (d, H1 and H10), 7 35 (d, H2 and H9), 8 20 (s, H4 and H7), 8 00 (s, H5 and H6)

Tjipanazole E $[\alpha]_D$ +63 8° (1 1 $CHCl_3$/MeOH, c 1 0), UV (MeOH) λ_{max} nm (ε) 259 (54,400), 293 (21,700), 332 (26,900), 350 (7,360), 368 (4,460), FDMS m/z (rel intensity) 486 (100), 488 (60), 490 (20), high resolution FABMS m/z 486 1067 (calcd for $C_{24}H_{20}N_2O_5Cl_2$, 486 0750) ^{1}H and ^{13}C NMR (9 1 $CDCl_3$/MeOH-d_4 containing a small amount of benzene-d_6) see Tables 1 and 2, respectively

Tjipanazole F compounds $[\alpha]_D$ +14 9° (1 1 $CHCl_3$/MeOH, c 1 0), UV (1 1 $CHCl_3$/MeOH) λ_{max} nm (ε) 258 (45,400), 268 (41,100), 290 (24,000), 327 (26,400), 361 (4,610), FDMS m/z (rel intensity) 422 (100), 423 (100), high resolution FABMS m/z 423 1067 (MH^+, calcd for $C_{23}H_{20}N_2O_4Cl$, 423 1112) **Tjipanazole F1** ^{1}H NMR (DMSO-d_6, 100 °C) δ 10 6 (br, 2H), 8 18 (d, 2 1 Hz), 8 13 (d, 7 Hz), 7 98 (d, 8 4 Hz), 7 94 (d, 8 4 Hz), 7 78 (d, 8 4 Hz), 7 70 (d, 8 4 Hz), 7 38 (t, 7 Hz), 7 38 (dd, 8 4 and 2 Hz), 7 22 (t, 7 7 Hz), 6 02 (d, 8 Hz), 4 87 (br), 4 70 (br, 2H), 4 23 (br), 3 89 (br, 2H), 3 76 (t, 10 5 Hz), 3 64 (t) **Tjipanazole F2** ^{1}H NMR (DMSO-d_6, 100 °C) δ 10 6 (br, 4H), 8 18 (d, 2 1 Hz), 8 14 (d, 7 Hz), 7 98 (d, 8 4 Hz), 7 95 (d, 8 4 Hz), 7 78 (d, 8 4 Hz), 7 71 (d, 8 4 Hz), 7 40 (t), 7 36 (dd, 8 4 and 2 Hz), 7 21 (t, 7 7 Hz), 6 00 (d, 8 Hz), 4 87 (br), 4 70 (br, 2H), 4 23 (br), 3 89 (br, 2H), 3 77 (t, 10 8 Hz), 3 64 (t)

Tjipanazole G compounds $[\alpha]_D$ +70 5° (1 1 $CHCl_3$/MeOH, c 0 6), UV (1 1 $CHCl_3$/MeOH) λ_{max} nm (ε) 256 (35,380), 270 (32,790), 287 (18,790), 324 (19,960), 343 (5,800), 359 (4,080), FDMS m/z (rel intensity) 402 (100), 403 (100), high resolution EIMS m/z 402 1587 (calcd for $C_{24}H_{22}N_2O_4$, 402 1580) **Tjipanazole G1** ^{1}H NMR (acetone-d_6) δ 7 55 (d, 8 1 Hz, H1), 7 35 (dd, 8 1 and 8 4 Hz, H2), 7 22 (t, 8 4 Hz, H3), 8 15 (d, 8 4 Hz, H4), 7 95 (d, 8 1 Hz, H5), 8 02 (d, 8 1 Hz, H6), 8 15 (d, 8 1 Hz, H7), 7 18 (t, 8 4 Hz, H8), 7 41 (t, 8 4 Hz, H9), 7 67 (d, 8 4 Hz, H10), 11 29 (s, 12NH), 6 29 (d, 6 3 Hz, H1'), 4 66 (m, H2'), 4 37 (m, H3'), 4 09 (m, H4'), 4 68 (q, 6 6 Hz, H5'), 1 41 (d, 6 6 Hz, H3-6') **Tjipanazole G2** ^{1}H NMR (acetone-d_6) δ 7 50 (d, 8 1 Hz, H1), 7 35 (dd, 8 1 and 8 4 Hz, H2), 7 22 (t, 8 4 Hz, H3), 8 15 (d, 8 4 Hz, H4), 7 95 (d, 8 1 Hz, H5), 8 02 (d, 8 1 Hz, H6), 8 15 (d, 8 1 Hz, H7), 7 18 (t, 8 4 Hz, H8), 7 41 (t, 8 4 Hz, H9), 7 67 (d, 8 4 Hz, H10), 11 13 (s, 12NH), 6 48 (d, 6 6 Hz, H1'), 4 73 (m, H2'), 4 43 (m, H3'), 4 28 (m, H4'), 4 58 (q, 7 2 Hz, H5'), 1 76 (d, 7 2 Hz, H3-6')

Tjipanazole I UV (1 1 $CHCl_3$/MeOH) λ_{max} nm (ε) 259 (49,600), 268 (48,500), 289 (28,400), 329 (27,200), 361 (4,430), FDMS m/z (rel intensity) 290 (100), 291 (100), high resolution FABMS m/z 291 0663 (MH^+, calcd for $C_{18}H_{12}N_2Cl$, 291 0690) ^1H NMR (acetone-d_6) δ 7 595 (d, 8 8 Hz, H1), 7 330 (dd, 8 8 and 2 1 Hz, H2), 8 160 (d, 2 1 Hz, H4), 7 952 (s, H5 and H6), 8 150 (dd, 8 0 and 1 0 Hz, H7), 7 202 (m, 8 0, 6 9 and 0 6 Hz, H8), 7 362 (m, 8 2, 6 9 and 1 0 Hz, H9), 7 587 (dd, 8 2 and 0 6 Hz, H10), NH signals not observed

Tjipanazole J UV (1 1 $CHCl_3$/MeOH) λ_{max} nm (ε) 238 (11,000), 259 (8,570), 303 (20,200), 339 (4,610), 369 (2,410), FDMS m/z (rel intensity) 395, 397, high resolution FABMS m/z 396 0306 (MH^+, calcd for $C_{20}H_{11}N_3OCl_2$, 396 0300) ^1H NMR (acetone-d_6) δ 9 17 (d, 2 4Hz), 8 84 (br s, NH), 8 34 (d, 2 4 Hz), 7 79 (d, 8 3 Hz), 7 76 (d, 8 3 Hz), 7 47 (dd, 8 3 and 2 4 Hz), 7 44 (dd, 8 3 and 2 4 Hz), 6 42 (s, 1H) ^{13}C NMR (DMSO-d_6) δ 170 62 (s, C-5), 138 03 (s), 137 89 (s), 135 71 (s, C-7a), 128 84 (s), 126 90 (s), 125 18 (d), 125 13 (d), 123 98 (s), 123 89 (d), 123 58 (s), 123 29 (s, 2C), 122 10 (d), 118 47 (s, C-4c), 114 60 (s), 114 52 (s), 113 44 (d), 113 20 (d), 78 48 (d, C-7)

Uniform C-13 and N-15 Enrichment of Tjipanazoles A1, A2 and B. *Tolypothrix tjipanasensis* DB-1-1 was grown in a 10 L glass vessel containing 8 L of an inorganic medium from which buffer had been omitted and 4 0 g $Na^{15}NO_3$ (99 atom %) added as the sole nitrogen source The culture was stirred, incubated at 24±2°C, illuminated at an incident intensity of 150 μEinstein m^{-2} s^{-1} with cool-white fluorescent lighting for a continuous period of 16 h per day, and aerated at approximately 1 L/min with ordinary air (no extra CO_2 added) The culture vessel was equipped with acid (0 5 N HCl) and base ($NaH^{13}CO_3$ solution) addition ports and an autoclavable pH electrode The pH was kept at 7 85±0 05 by continuous monitoring with a pH controller and automatic addition of acid A 1 L aqueous solution of 6 5 g $NaH^{13}CO_3$ (99 atom %) was added continuously over 26 days After 28 days, the 8 L culture (medium and cells) was lyophilized and the solid residue extracted twice with 1 L ethanol/water (7 3) for 12 h Workup as described above resulted in the isolation of ^{13}C,^{15}N labeled tjipanazoles A1/A2 (50 mg) and B (10 mg) Inspection of the ^{13}C NMR spectra of the labeled tjipanazoles indicated uniform ^{13}C enrichment to about 35% and ^{15}N enrichment to over 90% Analysis of the INADEQUATE spectra of these samples allowed the unambiguous assignments of the carbon signals for tjipanazoles A1, A2 and B (Table 1)

Acid Solvolysis of Tjipanazoles A1, A2, B and E A mixture of tjipanazoles A1 and A2 (35 mg) in 15 mL of 2N methanolic HCl was refluxed for 2 h under nitrogen Upon cooling tjipanazole D (23 mg) precipitated and was collected by filtration The filtrate was lyophilized and the resulting mixture of 6-deoxygulose and rhamnose (8 3 mg) was treated with 9% methanolic HCl to give the methyl glycosides Benzoylation with benzoyl chloride (1 mL) in pyridine (5 mL) gave the tribenzoylated methyl glycosides which could be separated into two components by repetitive TLC on silica gel, using successively 20% EtOAc and 30% EtOAc in petroleum ether Methyl-β-D-6-deoxygulopyranoside 2,3,4-tribenzoate $[\alpha]_D$ +40° ($CHCl_3$, c 1 0), CD (MeOH) $\Delta\varepsilon_{221}$ (+11), $\Delta\varepsilon_{237}$ (-3), UV (MeOH) λ_{max} nm (ε) 229 5 (45,200), ^1H NMR ($CDCl_3$) δ 5 88 (t, J = 3 5 Hz, H-3), 5 53 (dd, J = 8 4 and 3 5 Hz, H-2), 5 39 (dd, J = 3 5 and 1 5 Hz, H-4), 5 05 (d, J = 8 4 Hz, H-1), 4 48 (qd, J = 7 and 1 5 Hz, H-5), 3 63 (s, OMe), 1 38 (d, J = 7 Hz, H3-6), benzoate chemical shifts not determined, FDMS m/z 491 (MH^+), high resolution FABMS m/z 491 1662 (calcd for $C_{28}H_{27}O_8$, 491 1706) Methyl-α-L-rhamnopyranoside 2,3,4-tribenzoate $[\alpha]_D$ +171° ($CHCl_3$, c 1 5), CD (MeOH) $\Delta\varepsilon_{221}$ (-17), $\Delta\varepsilon_{237}$ (+64), identical with $[\alpha]_D$ and CD spectrum of an authentic sample prepared from L-rhamnose

A solution of tjipanazole B (50 mg) in 2N methanolic HCl was refluxed for 2 h to give 12 mg of methyl xyloside and 27 g of tjipanazole D after chromatography Acetylation of this material (acetic anhydride and pyridine) gave a gum that was a mixture of α and β-methyl-D-xyloside triacetate Purification by repeated preparative TLC on silica

gel gave 2 1 mg of the α anomer which had the same proton NMR spectrum in acetone-d_6 and the same optical rotation, $[\alpha]_D$ = +113 2° ($CHCl_3$, c 0 07), as reported[10,11] for α-methyl-D-xyloside triacetate

A solution of tjipanazole E (0 5 mg) in 2N methanolic HCl was refluxed for 4 h and evaporated to dryness The residue was extracted with water and the presence of D-glucose was detected semiquantitatively in the extract with Lilly Tes-Tape (color change from yellow to green)

Methyl-α-L-rhamnopyranoside 2,3,4-Tribenzoate. α-L-Rhamnose monohydrate (0 49 g) was dissolved in 1 5 N methanolic HCl and heated to reflux for 3 h The mixture was cooled and concentrated to give a residual gum which was dissolved in pyridine The solution was chilled to 0° and excess benzoyl chloride was added After stirring for 18 h at 25°, MeOH was added and after 10 min the reaction mixture was concentrated to an oil which was extracted with ether The ether-soluble material was purified by preparative HPLC on silica using a hexane to 1 3 hexane/EtOAc gradient The product was obtained as a white solid (287 mg), $[\alpha]_D$ +173° (c 1 5, $CHCl_3$), UV λ_{max} 230 nm (ε 37,100), 274 (3,810), 281 (2,280), ^{1}H NMR ($CDCl_3$) δ 5 86 (dd, J = 9 9 and 3 6 Hz, H-3), 5 70 (t, J = 9 9 Hz, H-4), 5 69 (dd, J = 3 6 and 1 6 Hz, H-2), 4 94 (d, J = 1 6 Hz, H-1), 4 21 (dq, J = 6 0 and 9 9 Hz, H-5), 3 52 (s, OMe), 1 40 (d, J = 6 0 Hz, Me on C-5), benzoate signals not measured, FDMS m/z 491 (MH+) Anal Found C, 68 48, H, 5 43 Calcd for $C_{28}H_{26}O_8$ C, 68 56, H, 5 34

Synthesis of Tjipanazole D To a solution of 32 3 g of p-chlorophenylhydrazine hydrochloride and 0 5 mL conc H_2SO_4 in 500 mL EtOH was added 9 9 g of 1,2-cyclohexanedione over 10 min The resulting mixture was slowly heated to 65° and after 45 min a precipitate began to appear The mixture was then cooled and stirred for 4 h The precipitate was separated by filtration, washed with cold EtOH and dried to give 24 3 g (72%) of the p-chlorophenylhydrazone of 1-oxo-1,2,3,4-tetrahydro-6-chlorocarbazole (Scheme 1), mp 190-193° (dec), UV (MeOH) λ_{max} nm (ε) 359 (40,300), 278 (7700), 228 (24,900), ^{1}H NMR (DMSO-d_6) δ 11 1 (s, indole NH), 7 50 (d, J = 1 Hz, H-5), 7 36 (d, J = 8 Hz, H-8), 7 35 (d, H-2' and H-6'), 7 25 (d, H-3' and H-5'), 7 10 (dd, H-7), 2 80 and 2 65 (m, H_2-2 and H2-4), 2 00 (m, H_2-3), FDMS m/z 343, 345

The hydrazone hydrochloride (6 6 g) was dissolved in 70 mL of glacial HOAc and refluxed for 12 h The resulting precipitate was dried to give 4 5 g (75%) of tjipanazole D, mp 320° (dec) Anal Found C, 66 30, H, 3 01, N, 8 35 Calcd for $C_{18}H_{10}N_2Cl_2$ C, 66 48, H, 3 10, N, 8 61

Synthesis of Tjipanazoles E and G2 To 1 0 g of tjipanazole D in 20 mL MeCN was added 0 25 g of 60% NaH After 1 h at room temperature, 1-bromo-α-D-glucopyranosyl 2,3,4,6-tetraacetate (1 91 g) was added in small portions, the mixture was heated to 50° for 5 h, cooled and filtered, and the filtrate was evaporated The residual solid was deesterified by treatment with 20 mL of 1 1 sat'd NH_3 in MeOH/MeOH at room temperature for 18 h The saponified material was subjected to preparative HPLC purification as described above to give 23 mg of tjipanazole E which was identical in all respects including optical rotation, $[\alpha]_D$ = +59 6° (1 1 $CHCl_3$/MeOH, c 1 0), with the natural product

To 128 mg of indolo[2,3-a]carbazole[15] in 2 mL MeCN was added 30 mg of 60% NaH After 1 h at room temperature, 1-bromo-α-L-rhamnopyranosyl 2,3,4-triacetate[16] (0 40 g) was added and the reaction allowed to proceed as described above The crude adduct was saponified using NH_3/MeOH and purified by preparative HPLC to afford 3 2 mg of tjipanazole G2 which was identical in all respects including optical rotation, $[\alpha]_D$ +66 7° (1 1 $CHCl_3$/MeOH, c 2 4), with the natural product

Using essentially the same procedure tjipanazole A2, $[\alpha]_D$ = +26 14° ($CHCl_3$, c 1 0), and tjipanazole B, $[\alpha]_D$ =

+10 9° (1 1 $CHCl_3$/MeOH, c 1 0), were synthesized, albeit in low yield, from 1-bromo-α-L-rhamnopyranosyl 2,3,4-tribenzoate[17] and 1-bromo-α-D-xylopyranosyl 2,3,4-triacetate,[18] respectively

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