Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 191–197
Carbohydrate RESEARCH

Synthesis of the pentasaccharide repeating unit of latosillan

Yuxia Hua,[a] Junjun Xiao,[b] Yingshen Huang[b] and Yuguo Du[a,*]

[a]*State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*
[b]*Department of Cell Biology, School of Basic Medical Sciences, Peking University, Beijing 100083, China*

Received 25 October 2005; received in revised form 9 November 2005; accepted 14 November 2005
Available online 5 December 2005

Abstract—A pentasaccharide, β-D-Man-(1→2)-[β-D-GlcNAc-(1→4)]-α-L-Rha-(1→4)-α-L-Rha-(1→4)-α-L-Rha-1-OC_8H_{17}, representing the repeating unit of latosillan, was convergently synthesized from the building blocks, ethyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside, 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate, and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate under standard glycosylation conditions. The target pentasaccharide showed acceptable differentiation-inducing activity on HL-60 cell lines at the dosages of 10–50 μg/mL.

Keywords: Glycosylation; Latosillan; Cell differentiation; Oligosaccharides

1. Introduction

The mouse myeloid leukemia cell line M1 was originally established in vitro from a spontaneous leukemia SL strain in the mouse.[1] It has been shown that M1 cells can be induced to differentiate into macrophages and granulocytes when treated with proteinous factors (D-factors) in conditioned media from various cells and in various body fluids, and with chemicals such as glucocorticoid hormones, 1α,25-dihydroxyvitamin D, and lipopolysaccharides.[1–3] In the screening course for differentiation inducer of M1 cells, Ando and his co-workers[4] have isolated a polysaccharide (named as latosillan later) from the culture filtrate of a bacterium, and a strong differentiation inducer activity was observed when incubated with M1 cells. The structure of latosillan was elucidated, from a degradation study and NMR spectral analysis, to be a heteroglycan composed of the repeating units of a pentasaccharide,[5] →2)-β-D-Man-(1→2)-[β-D-GlcNAc-(1→4)]-α-L-Rha-(1→4)-α-L-Rha-(1→4)-α-L-Rha-(1→, as shown in Figure 1.

To have a better understanding of this immunologically interesting observation and to compare the bioactivities between natural polysaccharide and the

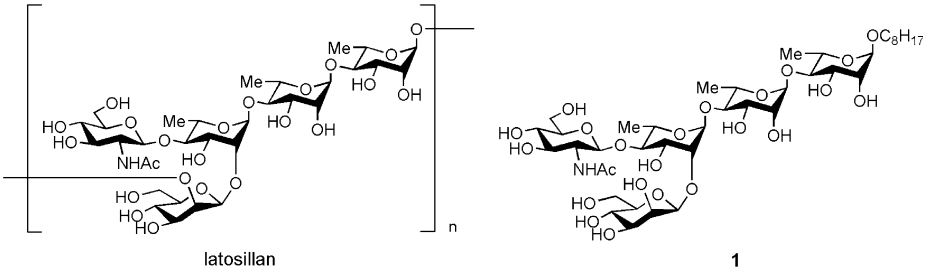


Figure 1. Structures of latosillan and compound **1**.

* Corresponding author. Tel.: +86 10 62914475; fax: +86 10 62923563; e-mail: duyuguo@mail.rcees.ac.cn

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.11.007

structural repeating unit, we launched a collaborative project regarding the preparation and potential medical application of latosillan-related analogues. Here, we would like to report the synthesis and preliminary biological studies of a latosillan pentasaccharide derivative.

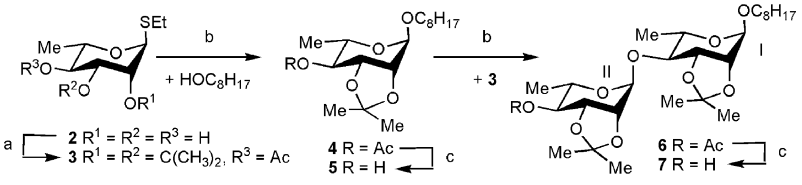
2. Results and discussion

Pentasaccharide **1** was prepared via a convergent '3+2' strategy. The synthesis of disaccharide acceptor **7** is described in Scheme 1. Ethyl 4-*O*-acetyl-2,3-*O*-isopropylidene-1-thio-α-L-rhamonopyranoside (**3**)[6] was converted into its octyl glycoside **4** under standard NIS/TMSOTf-catalyzed glycosylation conditions. Zemplén deacetylation[7] of **4** with NaOMe in MeOH furnished octyl 2,3-*O*-isopropylidene-α-L-rhamonopyranoside (**5**) in a yield of 86% for two steps. Glycosylation of **3** and **5** as described in the preparation of **4** gave octyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamonopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamonopyranoside (**6**), which was further deacetylated with NaOMe in MeOH furnishing disaccharide acceptor, octyl 2,3-*O*-isopropylidene-α-L-rhamonopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**7**) in 83% isolated yield for two steps. It is noteworthy that the chemical shift of H-1^{II} appears at δ 5.60 ppm (H-1^{I} at δ 4.95 ppm) in the ^{1}H NMR spectrum of **7**, a significant difference compared to α-(1→3) linked rhamnopyranosyl disaccharide (around δ 5.0 ppm).[8]

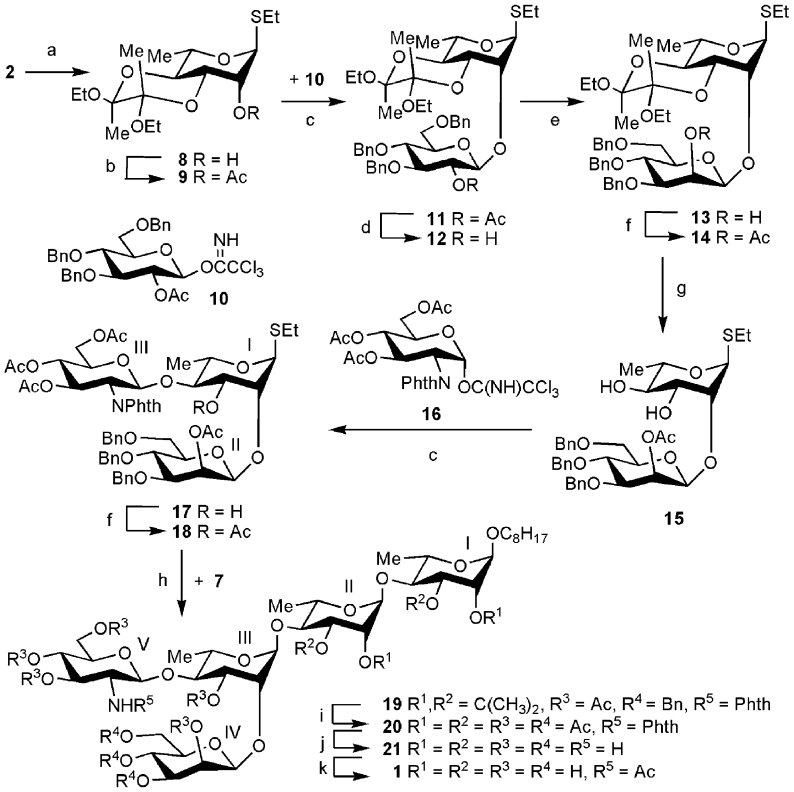
In our initial synthesis of trisaccharide donor, we expected to establish a properly protected β-D-GlcNAc-(1→4)-α-L-Rha residue first, then attach the 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl residue to the 2-OH of the above rhamnose unit, followed by O-deacetylation–oxidation–reduction on C-2 of the glucose unit to furnish a β-D-mannose containing trisaccharide. However, the multistep reactions finally gave an inseparable mixture having both β-D-GlcNAc-(1→4)-[β-D-Man-(1→2)]-α-L-Rha and β-D-GlcNAc-(1→4)-[β-D-Glc-(1→2)]-α-L-Rha in a ratio of about 4:1. We thus modified our strategy towards the synthesis of trisaccharide donor **18**, as outlined in Scheme 2.

Treatment of rhamnopyranosyl thioglycoside **2** with butanedione, triethyl orthoformate, and TsOH in EtOH furnished compound **8** with its *trans*-OHs blocked in 79% yield.[9] To prove this regioselectivity, **8** was acetylated with acetic anhydride in pyridine generating **9**, which provided a chemical shift of H-2 at 5.13 ppm ($J = 3.2$, 1.4 Hz) in the ^{1}H NMR spectrum, confirming the structure of **8**. Coupling of **8** and 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate (**10**)[10] in the presence of a catalytic amount of TMSOTf in CH_2Cl_2 at 0 °C gave ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4-*O*-(2′,3′-diethoxybutane-2′,3′-diyl)-1-thio-α-L-rhamnopyranoside (**11**) in 86% yield. Zemplén deacetylation of **11**, followed by oxidation with DMSO/Ac_2O,[11] reduction with $NaBH_4$, and acetylation with Ac_2O in pyridine, afforded ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4-*O*-(2′,3′-diethoxybutane-2′,3′-diyl)-1-thio-α-L-rhamnopyranoside (**14**) in 62% yield for four steps. In compound **11**, the chemical shifts of H-1′ and H-2′ appear at 4.58 ppm (d, $J = 8.4$ Hz) and 5.07 ppm (t, $J = 8.4$ Hz), while in **14**, H-1′ and H-4′ appear at 5.10 ppm ($J < 1.0$ Hz) and 5.76 ppm (d, $J = 2.6$ Hz), respectively. This indicated a successful transformation of the glucose derivative into the corresponding mannose derivative.[12] Hydrolysis of **14** with aqueous 90% TFA (→**15**), followed by regioselective 4-OH glycosylation with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**16**)[13] (→**17**) and acetylation with Ac_2O in pyridine, delivered trisaccharide donor ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**18**) in an overall yield of 55% for three steps. The characteristic peaks corresponding to H-1^{III}, H-4^{I}, and H-3^{I} in the ^{1}H NMR spectrum of **18** that appear at 5.48 ppm (d, $J = 8.3$ Hz), 3.79 ppm (t, $J = 9.5$ Hz), and 4.73 ppm (dd, $J = 9.5$, 3.1 Hz), respectively, further confirmed the desired selectivity on C-4 in the above glycosylation.

Condensation of trisaccharide donor **18** and disaccharide acceptor **7** in CH_2Cl_2, using NIS/TMSOTf as co-catalyst, stereoselectively gave pentasaccharide **19** in good isolated yield. 2D NMR spectra of **19** clearly showed 5 H-1s (H-1^{I}: 4.94 ppm; H-1^{II}: 5.52 ppm; H-1^{III}: 5.29 ppm; H-1^{IV}: 4.36 ppm; H-1^{V}: 5.47 ppm) and 5 C-1s (C-1^{I}: 96.80 ppm; C-1^{II}: 95.47 ppm; C-1^{III}: 97.58 ppm; C-1^{IV}: 100.04 ppm; C-1^{V}: 97.37 ppm), which are consistent with the desired structure. No β isomer was isolated from this reaction. Acetal cleavage of compound **19** was smoothly conducted with 90% aqueous acetic acid under reflux, and the intermediate was subse-



Scheme 1. Synthesis of disaccharide acceptor **7**. Reagents and conditions: (a) 2,2,-dimethoxypropane, acetone, TsOH, rt, 91%; (b) NIS, TMSOTf, CH_2Cl_2, −20 °C; (c) NaOMe, MeOH, rt, 86% for **5** from **3**, 83% for **7** from **4**.



Scheme 2. Synthesis of pentasaccharide **1**. Reagents and conditions: (a) triethyl orthoformate, butanedione, TsOH, EtOH, rt, 79%; (b) Ac_2O, Py; (c) TMSOTf, CH_2Cl_2, 0 °C, 86% for **11**, 65% for **17** at −60 °C; (d) NaOMe, MeOH, rt, 83%; (e) Ac_2O, DMSO, rt; $NaBH_4$, 1:1 CH_2Cl_2–MeOH, 0 °C; (f) Ac_2O, Py, 75% for **14** (from **12**), 100% for **18**; (g) 90% TFA aqueous solution, rt, 85%; (h) NIS/TMSOTf, −20 °C, 80%; (i) 90% AcOH aq, reflux; 20% $Pd(OH)_2/C$, H_2; Ac_2O, Py; (j) NH_3, 4:1 MeOH–CH_2Cl_2, 6 days; (k) Ac_2O, Py; NaOMe, MeOH, 59% based on **19**.

quently debenzylated with H_2 and $Pd(OH)_2/C$. The above residue was further treated with NH_3-saturated MeOH for 6 days to deprotect acetyl and phthalyl protecting groups. Global N,O-acetylation with acetic anhydride in pyridine, followed by O-deacetylation with NaOMe in MeOH, furnished pentasaccharide derivative **1** as a white foam in 59% yield from **19**.

The differentiation-inducing activity of pentasaccharide **1** in the HL-60 cell line was preliminarily studied according to the published method.[4,14] The results are summarized in Table 1. Our experiments indicate that compound **1** can induce NBT reduction and is a dose-dependent differentiation inducer for HL-60 cells. At a dosage of 50 μg/mL, compound **1** showed the same inducing activity as the commonly used positive control (ATRA).

Table 1. Compound **1** induced differentiation activities of HL-60 cells

Concentrations		NBT reduction ($A_{560\,nm}/10^6$cells)[a]
DEME	0	0.093 ± 0.025[b]
ATRA (μmol/L)	1	0.191 ± 0.006[c]
	1	0.095 ± 0.003[b,c]
Compound **1** (μg/mL)	10	0.130 ± 0.003[b,c]
	20	0.167 ± 0.005[b,c]
	50	0.212 ± 0.003[b,c]

[a] Data are presented as means ± SD from three separate experiments; ρ-values are calculated using one-factor analysis of variance with one-way ANOVA.
[b] $\rho^* < 0.01$ compared with the negative control.
[c] $\rho^* < 0.01$ compared with the positive control.

In conclusion, we have prepared a pentasaccharide derivative representing the natural latosillan repeating unit. In this convergent synthesis, a β-D-mannose unit was introduced by oxidation–reduction of a β-D-glucosyl 2-OH group using Ac_2O/DMSO–$NaBH_4$ conditions. L-Rhamnosyl thioglycosides were used as donors and provided good stereo outcomes in generating the α-glycosidic bond in NIS/TMSOTf-catalyzed glycosylations. The compound prepared showed acceptable differentiation-inducing activity on HL-60 cell lines at the dosages of 10–50 μg/mL. These results should be valuable in cell differentiation-related SAR studies. Synthesis and bioactivity studies regarding this pentasaccharide linear oligomer and dendrimer are currently under investigation in our group.

3. Experimental

3.1. General

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter, and

$[\alpha]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or CD_3OD. Chemical shifts are given in parts per million downfield from internal Me_4Si. Mass spectra were measured using a MALDITOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by UV detector. Column chromatography was conducted by elution of a column of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

3.2. Octyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (5)

To a solution of compound **3** (5.80 g, 20.00 mmol) and 1-octanol (2.90 mL, 18.26 mmol) in dry CH_2Cl_2 (50 mL) was added 4 Å molecular sieves (3 g). The mixture was stirred at −20 °C for 20 min under an N_2 atmosphere, then *N*-iodosuccinimide (7.35 g, 30.00 mmol) and TMSOTf (100 μL, 0.55 mmol) were added. The mixture was stirred under these conditions for 30 min, quenched by Et_3N, diluted with CH_2Cl_2 (100 mL), and washed with water (2 × 20 mL). The organic phase was dried over Na_2SO_4 and concentrated under diminished pressure to give crude product, which was subsequently dissolved in MeOH (100 mL). To this mixture was added NaOMe (1.0 M, kept pH at 9–10) at rt, stirred under these conditions for 3 h, then neutralized with Amberlite IR-120 (H^+). The mixture was filtered, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (4:1 petroleum ether–EtOAc) to give **5** as a white foam (5.44 g, 86%): $[\alpha]_D^{25}$ −49 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.98 (s, 1H, H-1), 4.19 (dd, 1H, *J* 3.5, 1.7 Hz, H-2), 4.14 (dd, 1H, *J* 10.0, 3.5 Hz, H-3), 3.76–3.85 (m, 2H, H-4, H-5), 3.66, 3.42 (2dt, 2H, *J* 6.5, 9.7 Hz, OCH_2), 1.59–1.57 (m, 2H, OCH_2CH_2), 1.55, 1.34 (2s, 6H, $(CH_3)_2C$), 1.29–1.26 (m, 10H, $5CH_2$), 1.16 (d, 3H, *J* 6.3 Hz, H-6), 0.89 (t, 3H, *J* 7.0 Hz, CH_3). Anal. Calcd for $C_{17}H_{32}O_5$: C, 64.53; H, 10.19. Found: C, 64.28; H, 10.25.

3.3. Octyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (6)

To a solution of compounds **3** (1.60 g, 5.5 mmol) and **5** (1.58 g, 5.0 mmol) in dry CH_2Cl_2 (20 mL) was added 4 Å molecular sieves (3 g). The mixture was stirred at −20 °C for 20 min under an N_2 atmosphere, then *N*-iodosuccinimide (2.02 g, 8.25 mmol) and TMSOTf (50 μL, 0.28 mmol) were added. The mixture was stirred under these conditions for 30 min, quenched by Et_3N, and concentrated. The residue was purified by silica gel column chromatography (5:1 petroleum ether–EtOAc) to give **6** as a syrup (2.45 g, 90%): $[\alpha]_D^{25}$ −67 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (s, 1H, H-1′), 4.95 (s, 1H, H-1), 4.88 (dd, 1H, *J* 10.1, 7.9 Hz, H-4′), 4.21 (dd, 1H, *J* 7.1, 5.6 Hz, H-3), 4.19 (d, 1H, *J* 5.6 Hz, H-2), 4.17 (dd, 1H, *J* 7.9, 5.8 Hz, H-3′), 4.10 (d, 1H, *J* 5.8 Hz, H-2′), 3.76–3.60 (m, 2H, H-5, H-5′), 3.67, 3.41 (2dt, 2H, *J* 9.6, 6.6 Hz, OCH_2), 3.59 (dd, 1H, *J* 9.9, 7.1 Hz, H-4), 2.10 (s, 3H, CH_3CO), 1.60–1.58 (m, 2H, OCH_2CH_2), 1.55, 1.53, 1.36, 1.33 (4s, 4 × 3H, $2(CH_3)_2C$), 1.30–1.26 (m, 10H, $5CH_2$), 1.21, 1.14 (d, 2 × 3H, *J* 6.3 Hz, H-6, H-6′), 0.89 (t, 3H, *J* 7.0 Hz, CH_3). Anal. Calcd for $C_{28}H_{48}O_{10}$: C, 61.74; H, 8.88. Found: C, 62.02; H, 8.96.

3.4. Octyl 2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (7)

Removal of the acetyl group from compound **6** (2.40 g, 4.41 mmol), as described in the preparation of **5**, gave **7** as a white foam (2.03 g, 92%): $[\alpha]_D^{25}$ −6 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 (s, 1H, H-1′), 4.95 (s, 1H, H-1), 4.23 (dd, 1H, *J* 7.0, 5.6 Hz, H-3), 4.19 (d, 1H, *J* 5.6 Hz, H-2), 4.10 (d, 1H, *J* 5.7 Hz, H-2′), 4.01 (dd, 1H, *J* 7.5, 5.7 Hz, H-3′), 3.69–3.56 (m, 4H, H-4, H-4′, H-5, H-5′), 3.42–3.39 (m, 2H, OCH_2), 1.55, 1.53, 1.36, 1.33 (4s, 4 × 3H, $(CH_3)_2C$), 1.60–1.58 (m, 2H, CH_2), 1.32–1.28 (m, 10H, $5CH_2$), 1.27, 1.25 (2d, 2 × 3H, *J* 6.3 Hz, H-6, H-6′), 0.89 (t, 3H, *J* 7.7 Hz, CH_3). Anal. Calcd for $C_{26}H_{46}O_9$: C, 62.13; H, 9.22. Found: C, 61.89; H, 9.14.

3.5. Ethyl 3,4-*O*-(2′,3′-diethoxybutane-2′,3′-diyl)-1-thio-α-L-rhamnopyranoside (8)

To a solution of compound **2** (4.20 g, 20.17 mmol) in EtOH (50 mL) was added triethyl orthoformate (21.3 mL, 161 mmol), butanedione (5.3 mL, 60.39 mmol), and TsOH (pH 3) at rt. The mixture was stirred under these conditions for 1 h, quenched by Et_3N, and concentrated. The residue was purified by silica gel column chromatography (6:1 petroleum ether–EtOAc) to give foamy **8** (5.60 g, 79%). To confirm the structure, compound **8** (30 mg) was acetylated with Ac_2O (1 mL) in pyridine (2 mL) affording **9** as a syrup, quantitatively: $[\alpha]_D^{25}$ −5 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18 (d, 1H, *J* 1.4 Hz, H-1), 5.13 (dd, 1H, *J* 3.2, 1.4 Hz, H-2), 4.15 (m, 1H, H-5), 4.07 (dd, 1H, *J* 10.1, 3.2 Hz, H-3), 3.73 (t, 1H, *J* 10.1 Hz, H-4), 3.51–3.49 (m, 2 × 2H, $2OCH_2$), 2.64–2.62 (m, 2H, SCH_2), 2.13 (s, 3H, CH_3CO), 1.26–1.20 (m, 18H, H-6 and $5CH_3$). For **8**: Anal. Calcd for $C_{16}H_{30}O_6S$: C, 54.83; H, 8.63. Found: C, 55.01; H, 8.72.

3.6. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4-*O*-(2′,3′-diethoxybutane-2′,3′-diyl)-1-thio-α-L-rhamnopyranoside (11)

To a solution of compound **8** (1.76 g, 5.02 mmol) and compound **10** (3.84 g, 6.02 mmol) in dry CH_2Cl_2 (30 mL) was added 4 Å molecular sieves (3 g) at 0 °C under an N_2 atmosphere. The mixture was stirred under these conditions for 20 min, then TMSOTf (21 μL, 0.12 mmol) was added. The reaction mixture was stirred under these conditions for 1 h, quenched by Et_3N, and concentrated. The residue was purified by silica gel column chromatography (4:1 petroleum ether–EtOAc) to give **11** (3.56 g, 86%) as a white foam: $[\alpha]_D^{25}$ −29 (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.28 (m, 15H, Ph*H*), 5.42 (d, 1H, *J* 1.1 Hz, H-1), 5.07 (t, 1H, *J* 8.4 Hz, H-2′), 4.80, 4.77, 4.68, 4.56 (4d, 4H, *J* 10.2 Hz, 2PhCH_2), 4.58 (dd, 1H, *J* 8.4 Hz, H-1′), 4.55 (s, 2H, PhCH_2), 4.05–4.03 (m, 1H, H-5), 3.93 (dd, 1H, *J* 10.2, 3.0 Hz, H-3), 3.86 (dd, 1H, *J* 3.0, 1.1 Hz, H-2), 3.69–3.64 (m, 5H, H-4, H-3′, H-4′, H-5′, H-6a′), 3.48–3.44 (m, 5H, H-6b′, 2OCH_2CH$_3$), 2.55–3.53 (m, 2H, SCH_2CH$_3$), 2.15 (s, 3H, CH_3CO), 1.23–1.11 (m, 18H, H-6, 5CH_3). Anal. Calcd for $C_{45}H_{60}O_{12}S$: C, 65.51; H, 7.33. Found: C, 65.82; H, 7.24.

3.7. Ethyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→2)-3,4-*O*-(2′,3′-diethoxybutane-2′,3′-diyl)-1-thio-α-L-rhamnopyranoside (12)

Removal of the acetyl group from compound **11** (3.20 g, 3.88 mmol) as described in the preparation of **5** gave **12** as a white foam (2.52 g, 83%): $[\alpha]_D^{25}$ −17 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.28 (m, 15H, Ph*H*), 5.34 (s, 1H, H-1), 5.07, 4.86, 4.79, 4.56, 4.53, 4.50 (6d, 6H, *J* 10.2 Hz, PhCH_2), 4.42 (d, 1H, *J* 7.3 Hz, H-1′), 4.14–4.12 (m, 1H, H-5), 4.01 (dd, 1H, *J* 10.0, 2.9 Hz, H-3), 3.98 (d, 1H, *J* 2.9 Hz, H-2), 3.66–3.61 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.53–3.48 (m, 6H, H-6′, OCH_2CH$_3$), 2.62–2.59 (m, 2H, SCH_2CH$_3$), 1.23–1.11 (m, 18H, H-6, CH_3). MALDITOF-MS: calcd for $C_{43}H_{58}O_{11}S$, *m*/*z* 782; found: *m*/*z* 805.3 $(M+Na)^+$. Anal. Calcd for $C_{43}H_{58}O_{11}S$: C, 65.96; H, 7.47. Found: C, 66.25; H, 7.41.

3.8. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4-*O*-(2′,3′-diethoxybutane-2′,3′-diyl)-1-thio-α-L-rhamnopyranoside (14)

A solution of compound **12** (1.51 g, 1.93 mmol) in 1,2 Ac_2O–DMSO (15 mL) was kept at room temperature until all starting materials were consumed based on TLC monitoring. The mixture was diluted with CH_2Cl_2 (90 mL) and washed with water (3 × 40 mL). The organic layer was dried over $MgSO_4$ and evaporated. To the above-mentioned crude residue in 1:1 CH_2Cl_2–MeOH (20 mL) at 0 °C was added $NaBH_4$ (700 mg) in one portion. The reaction mixture was stirred at rt for 6 h, then diluted with CH_2C1_2, and the organic phase was successively washed with water, aq $NaHCO_3$, and brine. The organic solvent was evaporated in vacuum, and the crude residue was acetylated with Ac_2O (5 mL) in dry pyridine (10 mL) at rt for 12 h, then concentrated to dryness with the help of toluene. The residue was purified by silica gel column chromatography (5:1 petroleum ether–EtOAc) to yield **14** (1.20 g, 75% for three steps) as a white foam: $[\alpha]_D^{25}$ −52 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.28 (m, 15H, Ph*H*), 5.76 (br d, 1H, *J* 2.6 Hz, H-2′), 5.32 (d, 1H, *J* 1.1 Hz, H-1), 5.10 (br s, 1H, H-1′), 4.85, 4.80, 4.66, 4.58, 4.50, 4.47 (6d, 6H, *J* 10.2 Hz, PhCH_2), 4.16–4.14 (m, 1H, H-4′), 4.10–4.07 (m, 1H, H-5), 4.01 (dd, 1H, *J* 10.2, 2.6 Hz, H-3′), 3.86 (dd, 1H, *J* 3.0, 1.1 Hz, H-2), 3.69–3.64 (m, 5H, H-4, H-3, H-4′, H-5′, H-6a′), 3.49–3.44 (m, 5H, H-6b′, OCH_2), 2.57–2.54 (m, 2H, SCH_2), 2.15 (s, 3H, CH_3CO), 1.23–1.11 (m, 18H, H-6, 5CH_3). Anal. Calcd for $C_{45}H_{60}O_{12}S$: C, 65.51; H, 7.33. Found: C, 65.77; H, 7.29.

3.9. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-1-thio-α-L-rhamnopyranoside (15)

Compound **14** (1.15 g, 1.39 mmol) was dissolved in 90% aqueous TFA (8 mL), stirred at rt for 30 min, and co-evaporated with toluene to dryness under diminished pressure. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give **15** (810 mg, 85%) as a colorless syrup: $[\alpha]_D^{25}$ −35 (*c* 1.5, $CHCl_3$). To confirm the structure, compound **15** (50 mg, 0.07 mmol) was acetylated with Ac_2O (0.5 mL) in pyridine (1 mL) affording ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-3,4-di-*O*-acetyl-1-thio-α-L-rhamnopyranoside, quantitatively: $[\alpha]_D^{25}$ −13 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.28 (m, 15H, Ph*H*), 5.67 (br d, 1H, *J* 2.6 Hz, H-2′), 5.41 (d, 1H, *J* 1.4 Hz, H-1), 5.10 (dd, 1H, *J* 10.0, 3.1 Hz, H-3), 5.05 (dd, 1H, *J* 10.0, 9.4 Hz, H-4), 4.85, 4.80, 4.66, 4.58, 4.50, 4.47 (6d, 6 × 1H, *J* 10.2 Hz, PhCH_2), 4.61 (br s, 1H, H-1′), 4.26–4.24 (m, 1H, H-6a′), 4.14–4.11 (m, 1H, H-5), 3.74–3.67 (m, 3H, H-2, H-4′, H-6b′), 3.64 (dd, 1H, *J* 10.2, 2.6 Hz, H-3′), 3.44–3.42 (m, 1H, H-5′), 2.56–2.53 (m, 2H, SCH_2), 2.21, 2.06, 2.01 (s, 3 × 3H, 3CH_3CO), 1.23–1.11 (m, 6H, H-6, SCH$_2$CH_3). Anal. Calcd for $C_{37}H_{46}O_{10}S$ (compound **15**): C, 65.08; H, 6.79. Found: C, 64.80; H, 6.86.

3.10. Ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-3-*O*-acetyl-1-thio-α-L-rhamnopyranoside (18)

To a solution of compounds **15** (500 mg, 0.73 mmol) and **16** (465 mg, 0.80 mmol) in dry CH_2Cl_2 (10 mL) was

added 4 Å molecular sieves (1 g) at −60 °C under an N_2 atmosphere. The mixture was stirred under these conditions for 20 min, and then TMSOTf (10 μL, 0.06 mmol) was added and stirred for another 30 min, quenched by Et_3N. The mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to give **17** as a white foam, which was acetylated with Ac_2O (1 mL) in pyridine (3 mL) to furnish **18** (543 mg, 65% for two steps) as a syrup: $[\alpha]_D^{25}$ −42 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.90–7.18 (m, 19H, Ph*H*), 5.83 (dd, 1H, *J* 10.8, 9.0 Hz, H-3^{III}), 5.67 (br d, 1H, *J* 3.0 Hz, H-2^{II}), 5.48 (d, 1H, *J* 8.3 Hz, H-1^{III}), 5.28 (d, 1H, *J* 1.4 Hz, H-1^{I}), 5.20 (dd, 1H, *J* 10.0, 9.0 Hz, H-4^{III}), 4.87, 4.77, 4.57, 4.55, 4.53, 4.49 (6d, 6H, *J* 10.2 Hz, 3PhCH_2), 4.73 (dd, 1H, *J* 9.5, 3.1 Hz, H-3^{I}), 4.40 (dd, 1H, *J* 12.3, 3.7 Hz, H-$6a^{III}$), 4.37 (br s, 1H, H-1^{II}), 4.30 (dd, 1H, *J* 12.3, 3.7 Hz, H-$6b^{III}$), 4.23 (dd, 1H, *J* 10.8, 8.3 Hz, H-2^{III}), 4.03 (dd, 1H, *J* 1.4, 3.1 Hz, H-2^{I}), 3.98 (dt, 1H, H-5^{III}), 3.90–3.85 (m, 1H, H-5^{I}), 3.79 (t, 1H, *J* 9.5 Hz, H-4^{I}), 3.73 (t, 1H, *J* 9.5 Hz, H-4^{II}), 3.70 (d, 2H, *J* 3.6 Hz, H-6^{II}), 3.60 (dd, 1H, *J* 9.5, 3.0 Hz, H-3^{II}), 3.39–3.31 (dt, 1H, H-5^{II}), 2.51–2.46 (m, 2H, SCH_2), 2.21, 2.15, 2.06, 2.01, 1.88 (5s, 5 × 3H, 5CH_3CO), 1.24 (d, 3H, *J* 6.3 Hz, H-6^{I}), 1.15 (t, 3H, *J* 7.3 Hz, CH_3). Anal. Calcd for $C_{59}H_{67}NO_{20}S$: C, 62.04; H, 5.91. Found: C, 62.31; H, 5.80.

3.11. Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl-(1→2)]-3-*O*-acetyl-α-L-rhamnopyranoyl-(1→4)-4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (19)

Coupling of disaccharide **7** (200 mg, 0.40 mmol) and trisaccharide **18** (490 mg, 0.43 mmol) was carried out as described in the preparation of **6**. The crude product was purified on a silica gel column (2:1 petroleum ether–EtOAc) to yield **19** as a foamy solid (504 mg, 80%): $[\alpha]_D^{25}$ −100 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.78–7.18 (m, 19H, Ph*H*), 5.84 (dd, 1H, *J* 10.8, 9.0 Hz, H-3^{V}), 5.67 (br d, 1H, *J* 3.2 Hz, H-2^{IV}), 5.52 (s, 1H, H-1^{II}), 5.48 (d, 1H, *J* 8.4 Hz, H-1^{V}), 5.28 (d, 1H, *J* 1.6 Hz, H-1^{III}), 5.20 (dd, 1H, *J* 10.0, 9.0 Hz, H-4^{V}), 4.93 (s, 1H, H-1^{I}), 4.87, 4.80, 4.64, 4.57, 4.53, 4.48 (6d, 6 × 1H, *J* 10.8 Hz, 3 PhCH_2), 4.78 (dd, 1H, *J* 9.6, 3.2 Hz, H-3^{III}), 4.41 (dd, 1H, *J* 12.4, 3.0 Hz, H-$6a^{V}$), 4.37 (br s, 1H, H-1^{IV}), 4.30 (dd, 1H, *J* 12.4, 3.0 Hz, H-$6b^{V}$), 4.23 (dd, 1H, *J* 10.8, 8.4 Hz, H-2^{V}), 4.16 (dd, 1H, *J* 7.2, 5.6 Hz, H-3^{II}), 4.06 (d, 1H, *J* 5.6 Hz, H-2^{II}), 4.00–3.93 (m, 4H, H-2^{III}, H-3^{I}, H-5^{V}, H-$6a^{IV}$), 3.81 (t, 3H, *J* 9.6 Hz, H-4^{III}), 3.79 (t, 3H, *J* 10.0 Hz, H-4^{IV}), 3.75 (d, 1H, *J* 5.6 Hz, H-2^{I}), 3.70 (dd, 1H, *J* 1.2, 11.2 Hz, H-$6b^{IV}$), 3.68–3.50 (m, 6H, H-4^{I}, H-5^{I}, H-5^{II}, H-5^{III}, H-3^{IV}, OC*H*), 3.37–3.33 (m, 3H, H-4^{II}, H-5^{IV}, OC*H*), 2.20, 2.12, 2.10, 2.03, 1.85 (5s, 5 × 3H, 5 CH_3CO), 1.56, 1.42, 1.34, 1.19 (4s, 4 × 3H, 2(CH_3)$_2$C), 1.61–1.57 (m, 2H, OCH_2CH_2), 1.30–1.27 (m, 10H, 5CH_2), 1.20 (d, 6H, *J* 6.3 Hz, H-6^{I}, H-6^{III}), 1.14 (d, 3H, *J* 6.3 Hz, H-6^{II}), 0.89 (t, 3H, *J* 7.0 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.4, 170.2, 170.1, 169.5, 138.3, 138.1, 137.8, 137.4, 134.2, 129.0, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 109.3, 109.0, 100.0, 97.6, 97.4, 96.8, 95.4, 79.8, 78.5, 78.0, 77.5, 77.3, 77.0, 76.7, 75.8, 75.1, 74.6, 74.0, 73.6, 73.1, 71.4, 71.3, 70.6, 69.1, 69.0, 67.8, 67.6, 67.3, 64.5, 63.8, 61.6, 54.8, 31.8, 29.3, 29.2, 29.1, 27.9, 27.8, 26.3, 26.1, 22.6, 21.4, 21.2, 21.0, 20.7, 20.6, 20.6, 18.0, 17.7, 17.6, 14.0. MALDITOF-MS: calcd for $C_{83}H_{107}NO_{29}$, *m*/*z* 1581; found: *m*/*z* 1604.8 $(M+Na)^+$, 1620.8 $(M+K)^+$. Anal. Calcd for $C_{83}H_{107}NO_{29}$: C, 62.99; H, 7.81. Found: C, 63.28; H, 7.72.

3.12. Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-[β-D-mannopyranosyl-(1→2)]-α-L-rhamnopyranoyl-(1→4)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (1)

Compound **19** (410 mg, 0.26 mmol) was dissolved in 90% aq acetic acid (8 mL) and stirred under reflux for 30 min. At the end of this time, TLC indicated all starting material was consumed. The mixture was co-evaporated with toluene under diminished pressure to give a syrup, which was subjected to hydrogenation with H_2 under a flow rate of 100 mL/min in the presence of 20% $Pd(OH)_2$ on charcoal (209 mg, 0.14 mmol) in 1:1 MeOH–EtOAc (20 mL) for 70 h. The reaction mixture was filtered, the filtrate was concentrated, and the syrup was treated with Ac_2O (2 mL) in pyridine (4 mL) for 4 h at rt. After co-evaporation with toluene, the residue (about 320 mg) was dissolved into NH_3-saturated 4:1 MeOH–CH_2Cl_2 (50 mL) and stirred at rt for 6 days, then concentrated under diminished pressure. The residue was dissolved in H_2O (1 mL) and passed through a Sephadex LH-20 column with H_2O as eluent yielding foamy intermediate (about 175 mg) after freeze drying. Acetylation of this intermediate as described in the preparation of **14**, followed by purification on a silica gel column (3:2 petroleum ether–EtOAc) gave an amorphous solid. To a solution of the above solid in MeOH was added NaOMe (1.0 M, kept at pH 9–10) at rt. The reaction mixture was stirred for 3 h, then neutralized with Amberlite IR-120 (H^+), and filtered. The filtrate was concentrated, and the residue was purified on a Sephadex LH-20 column with H_2O as eluent to finish compound **1** (143 mg, 59% from **19**) as a white foam after freeze drying: $[\alpha]_D^{25}$ −32 (*c* 1, H_2O); Selected ^1H NMR (400 MHz, CD_3OD) δ 5.46 (d, 1H, *J* 1.2 Hz, H-1^{II}), 5.14 (d, 1H, *J* 1.6 Hz, H-1^{III}), 4.76 (d, 1H, *J* 8.3 Hz, H-1^{V}), 4.62 (br s, 2H, H-1^{I}, H-1^{IV}), 4.03 (dd, 1H, *J* 1.6, 3.4 Hz, H-2^{III}), 4.01 (d, 1H, *J* 2.7 Hz, H-2^{IV});^{13}C NMR (100 MHz, CD_3OD): δ

174.4, 103.7, 103.3, 102.9, 102.3, 101.4, 81.7, 81.3, 81.2, 80.9, 78.7, 77.8, 76.7, 75.2, 73.3, 73.2, 72.9, 72.7, 72.2, 72.0, 68.8, 68.5, 68.2, 63.1, 62.9, 58.2, 33.0, 30.5, 30.4, 27.3, 23.7, 23.0, 18.7, 18.4, 18.1, 14.5. MALDITOF-MS: calcd for $C_{40}H_{71}NO_{23}$, *m*/*z* 933; found: *m*/*z* 956.1 $(M+Na)^+$, 972.1 $(M+K)^+$. Anal. Calcd for $C_{40}H_{71}NO_{23}$: C, 51.44; H, 7.66. Found: C, 51.19; H, 7.79.

3.13. Bioassays of compound 1

M1 cells (3×10^5/well) were cultured in a suspension of Dulbcco's Modified Eagle Medium (DMEM) supplemented with 10% newborn calf serum in a humidified atmosphere at 37 °C containing 5% CO_2. Compound **1** was applied at concentrations of 10, 20, and 50 μg/mL according to the dosage applied for natural latosillan in the literature,[4] while positive control all-*trans*-retinoic acid (ATRA) was used at 1 μmol/L. DMEM medium was used as a negative control. At the end of 3 days of incubation, the cells were harvested by centrifugation, and then suspended in nitroblue tetrazolium (NBT) solution (100 μL, 4.0 mg/mL), and 12-*O*-tetradecanoylphorbol-13-acetate (TPA, 100 μL, 2.0 μg/mL) was added. The cell suspension was incubated at 37 °C for 20 min, and 1 N HCl (200 μL) was added at 4 °C to terminate the reaction. After centrifugation, DMSO (600 μL) was added to the cell pellets, and the amount of formazan formed in this process was measured at 560 nm with a microplate reader.

Acknowledgments

This work was supported by NNSF of China (Projects 20372081, 30330690).

References

1. Ichikawa, Y. *J. Cell. Physiol.* **1969**, *74*, 223–234.
2. Sachs, L. *Nature* **1978**, *274*, 535–539.
3. Abe, E.; Miyaura, C.; Sakagami, H.; Konno, K.; Yamazaki, T.; Yoshiki, S.; Suda, T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4990–4994.
4. Hayakawa, Y.; Ando, T.; Shimazu, A.; Seto, H.; Otake, N. *Agric. Biol. Chem.* **1985**, *49*, 2437–2442.
5. (a) Hayakawa, Y.; Nakagawa, M.; Ando, T.; Shimazu, A.; Seto, A.; Otake, N. *J. Antibiot.* **1982**, *35*, 1252–1254; (b) Hayakawa, Y.; Nakagawa, M.; Seto, A.; Otake, N. *Agric. Biol. Chem.* **1985**, *49*, 2443–2446.
6. (a) Yu, B.; Yu, H.; Hui, Y.; Han, X. *Tetrahedron Lett.* **1999**, *40*, 8591–8594; (b) van Steijn, A. M. P.; Jetten, M.; Kamerling, J. P.; Vliegenthart, J. F. G. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 374–383.
7. (a) Zemplén, G.; Kunz, A. *Ber. Dtsch. Chem. Ges.* **1923**, *56B*, 1705–1710; (b) Du, Y.; Pan, Q.; Kong, F. *Carbohydr. Res.* **2000**, *329*, 17–24.
8. (a) Reimer, K. B.; Harris, S. L.; Varma, V.; Pinto, B. M. *Carbohydr. Res.* **1992**, *228*, 399–414; (b) Marino-Albernas, J.-R.; Harris, S. L.; Varma, V.; Pinto, B. M. *Carbohydr. Res.* **1993**, *245*, 245–257.
9. (a) Chou, C.-H.; Wu, C.-S.; Chen, C.-H.; Lu, L.-D.; Kulkarni, S. S.; Wong, C.-H.; Hung, S.-C. *Org. Lett.* **2004**, *6*, 585–588; (b) Maloney, D. J.; Hecht, S. M. *Org. Lett.* **2005**, *7*, 1097–1099.
10. Du, Y.; Wei, G.; Linhardt, R. J. *J. Org. Chem.* **2004**, *69*, 2206–2209.
11. Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661–5670.
12. (a) Liu, K. K. C.; Danishefsky, S. *J. Org. Chem.* **1994**, *59*, 1892–1894; (b) Warren, C. D.; Augé, C.; Laver, M. L.; Suzuki, S.; Power, D.; Jeanloz, R. W. *Carbohydr. Res.* **1980**, *82*, 71–81; (c) Crich, D.; Sun, S. *Tetrahedron Lett.* **1998**, *39*, 1681–1684.
13. Du, Y.; Zhang, M.; Kong, F. *Tetrahedron* **2001**, *57*, 1757–1763.
14. Kohroki, J.; Muto, N.; Tanaka, T.; Itoh, N.; Inada, A.; Tanaka, K. *Leukemia Res.* **1998**, *22*, 405–412.