Pergamon

Tetrahedron Letters 43 (2002) 1477–1480

TETRAHEDRON LETTERS

The isolation and synthesis of two novel *N*-acetyl glucosamine derivatives from *Dictyostelium* cellular slime molds which exhibit neurite outgrowth activity

Haruhisa Kikuchi, Jun Komiya, Yoshinori Saito, Jun-ichi Sekiya, Shigeyoshi Honma, Norimichi Nakahata and Yoshiteru Oshima*

Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba-yama, Aoba-ku, Sendai 980-8578, Japan

Received 16 November 2001; accepted 21 December 2001

Abstract—To clarify the diversity of secondary metabolites of *Dictyostelium* cellular slime molds and explore any biologically active substances which they may hold, constituents of two species were investigated. From a methanol extract of their fruit bodies, two novel acylated amino sugars were isolated, dictyoglucosamine A (**1**) from *D. purpureum* and dictyoglucosamine B (**2**) from *D. discoideum*. For further analysis, these compounds were then synthesized from *N*-acetyl-D-glucosamine. Biological evaluation of **1** and **2** showed they induce neuronal differentiation of rat pheochromocytoma (PC-12) cells.

Cellular slime molds are unique organisms with a two stage life cycle: an initial unicellular animal-like feeding stage (a vegetable amoeba stage) and a multicellular plant-like fruit body stage (a reproductive fruit body stage).[1] Because their fruit bodies are small (ca. 2–3 mm in length), it is very difficult to obtain sufficient material from natural sources for research on their natural product chemistry. Fruit bodies of *Dictyostelium* cellular slime molds can also be supplied by the cultivation of spores with *Escherichia coli* on A-medium, however, the cultivation of a significant amount is tedious. Therefore, little attention has been paid to studying their secondary metabolites. Recently, we have cultured ca. 200 g of cellular slime molds and isolated novel secondary metabolites, such as biologically active α-pyrones, aromatics and amino sugar analogues.[2–5] The obtained compounds suggested that cellular slime molds may exhibit diversity in their secondary metabolite biosyntheses, and prompted us to pursue a variety of chemical constituents. In this paper, we describe the structure elucidation and syntheses of two novel acyl derivatives of *N*-acetyl glucosamine (**1** and **2**) as well as their potential ability to induce neurite outgrowth in rat pheochromocytoma (PC-12) cells.

Fruit bodies of the cellular slime mold, *D. purpureum*, were cultured on plates (wet weight: 189 g). They were extracted three times with methanol at room temperature to yield an extract (7.3 g), which was partitioned with ethyl acetate and water. The ethyl acetate soluble fraction (2.0 g) was separated by conventional column chromatography over silica gel and ODS to yield **1** (37.5 mg). In the same manner, **2** (0.5 mg) was afforded from fruit bodies of *D. discoideum* (wet weight: 109 g).

The molecular formula, $C_{29}H_{53}NO_8$, indicated for **1**,[6] was established by HREI-MS (m/z 544.3872 $[M+H]^+$) in addition to ^1H and ^{13}C NMR spectra (Table 1). The ^{13}C NMR spectrum of **1** revealed the presence of two ester carbonyls, an amide carbonyl, an acetal, three oxymethines, an oxymethylene, a nitrogen-adjacent methine, seventeen methylenes, and three methyl groups. The ^1H NMR spectrum and ^1H–^1H COSY of **1** gave the sequence of protons as follows (δ 5.24 (1H, br.t, $J=3.6$ Hz, H-1), 4.21 (1H, ddd, $J=10.8$, 9.4, 3.6 Hz, H-2), 5.22 (1H, dd, $J=10.8$, 9.6 Hz, H-3), 3.59 (1H, td, $J=9.6$, 5.6 Hz, H-4), 4.07 (1H, ddd, $J=9.6$, 3.9, 2.4 Hz, H-5), 4.46 (1H, dd, $J=12.2$, 3.9 Hz, H-6a) and 4.34 (1H, dd, $J=12.2$, 2.4 Hz, H-6b)), suggesting **1** is an analogue of an amino sugar. Coupling constants (J) of these protons ($J_{1,2}=3.6$ Hz, $J_{2,3}=10.8$ Hz, $J_{3,4}=9.6$ Hz and $J_{4,5}=9.6$ Hz) determined their spatial relationships from H-1 to H-5 as β, β, α, β and α, respectively. Thus, **1** is a derivative of 2-amino-2-deoxy-α-glucopyranose. In the HMBC spectrum, correlations of an amide car-

Keywords: cellular slime molds; amino sugars; differentiation; PC-12 cell.

* Corresponding author. Tel.: +81-22-217-6822; fax: +81-22-217-6821; e-mail: oshima@mail.pharm.tohoku.ac.jp

0040-4039/02/$ - see front matter
PII: S0040-4039(02)00038-2

Table 1. ^{13}C and ^{1}H NMR spectral data[a] of dictyoglucosamine A (**1**) and B (**2**)

Positions	Dictyoglucosamine A (**1**)		Dictyoglucosamine B (**2**)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	91.9	5.24 (1H, br.t, $J=3.6$ Hz)	91.8	5.23 (1H, br.t $J=3.4$ Hz)
2	52.1	4.21 (1H, ddd, $J=10.8$, 9.4, 3.6 Hz)	52.1	4.19 (1H, ddd, $J=10.8$, 9.3, 3.4 Hz)
3	72.9	5.22 (1H, dd, $J=10.8$, 9.6 Hz)	73.0	5.22 (1H, dd, $J=10.8$, 9.6 Hz)
4	68.8	3.59 (1H, td, $J=9.6$, 5.6 Hz)	68.8	3.59 (1H, td, $J=9.6$, 5.4 Hz)
5	70.1	4.07 (1H, ddd, $J=9.6$, 3.9, 2.4 Hz)	70.1	4.07 (1H, ddd, $J=9.6$, 3.8, 2.4 Hz)
6	62.9	4.46 (1H, dd, $J=12.2$, 3.9 Hz)	62.7	4.44 (1H, dd, $J=12.2$, 3.8 Hz)
		4.34 (1H, dd, $J=12.2$, 2.4 Hz)		4.34 (1H, dd, $J=12.2$, 2.4 Hz)
1′	170.4		170.4	
2′	23.1	1.95 (3H, s)	23.1	1.95 (3H, s)
1″	175.3[b]		175.3	
2″	34.3	2.35 (1H, dt, $J=15.6$, 7.8 Hz)	34.3	2.35 (1H, dt, $J=15.6$, 7.8 Hz)
		2.34 (1H, dt, $J=15.6$, 7.8 Hz)		2.34 (1H, dt, $J=15.6$, 7.8 Hz)
3″	25.0	1.56–1.62 (2H, m)	25.0	1.56–1.62 (2H, m)
4″–15″	[c]	1.20–1.32 (28H, m)	[c]	1.20–1.32 (28H, m)
16″	31.9	1.20–1.32 (28H, m)	31.9	1.20–1.32 (28H, m)
17″	22.7	1.20–1.32 (28H, m)	22.7	1.20–1.32 (28H, m)
18″	14.1	0.88 (3H, t, $J=6.8$ Hz)	14.1	0.88 (3H, t, $J=6.9$ Hz)
1‴	175.2[b]		174.0	
2‴	27.4	2.41 (2H, q, $J=7.5$ Hz)	43.2	2.26 (2H, d, $J=7.1$ Hz)
3‴	9.0	1.17 (3H, t, $J=7.5$ Hz)	25.7	2.07–2.15 (1H, m)
4‴			22.4	0.98 (6H, d, $J=6.7$ Hz)
1-OH		3.93 (1H, br.d, $J=3.6$ Hz)		4.02 (1H, br.d, $J=3.4$ Hz)
4-OH		3.01 (1H, d, $J=5.6$ Hz)		3.05 (1H, d, $J=5.4$ Hz)
2-NH		6.13 (1H, d, $J=9.4$ Hz)		6.16 (1H, d, $J=9.3$ Hz)

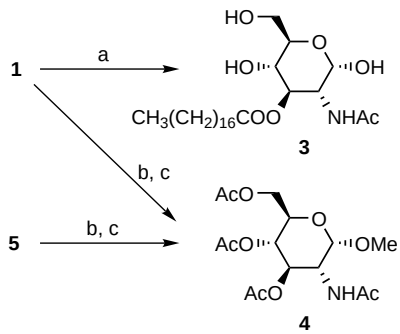
[a] 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$.
[b] These signals were indistinguishable.
[c] These carbon atoms were assigned to 12 signals: δ_c 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.3, 29.3, 29.3, 29.1.

bonyl carbon (δ_c 170.4) with the methyl protons (δ 1.95 (3H, s)), the nitrogen-adjacent methine proton (δ 4.21) and the amide proton (δ 6.13 (1H, d, $J=9.4$ Hz)) indicated the presence of an acetamide group at C-2. ^{1}H–^{1}H COSY pointed out the presence of a propionyloxy group. The presence of a stearoyloxy group was deduced from the remaining groups including an ester group, 16 methylene carbons and a methyl carbon. The positions of two ester groups remained unresolved as the correlational peaks of these carbonyl carbons (δ_c 175.3 and 175.2) were indistinguishable in the HMBC spectrum.

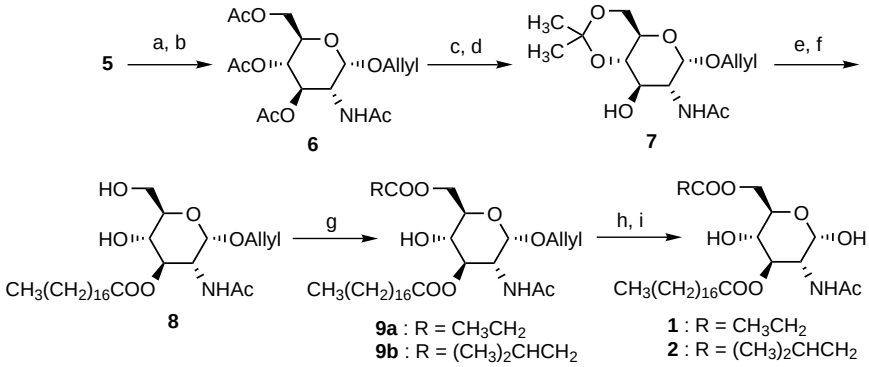
Methanolysis of **1** at room temperature gave the partially hydrolized product (**3**)[7] (Scheme 1). The upfield-shift of the signals of H-6 (δ 3.89 (1H, dd, $J=12.1$, 3.0 Hz) and 3.84 (1H, dd, $J=12.1$, 4.0 Hz)) in the ^{1}H NMR spectrum of **3** showed that the propionyloxy group of **1** was connected at C-6. The stearoyloxy group was thus located at C-3. Additionally, methanolysis of **1** in refluxing conditions followed by acetylation afforded the tetraacetyl derivative of methyl glycoside **4**. The specific rotation of **4** ($[\alpha]_D$ +68.5 (*c* 0.11, $CHCl_3$)) was identical to synthetically produced **4** ($[\alpha]_D$ +83.5 (*c* 0.17, $CHCl_3$)) obtained from *N*-acetyl-D-glucosamine (**5**). From this result, the absolute configuration of **1** was determined to be identical to *N*-acetyl-D-glucosamine.

The molecular formula of **2**[6] was determined by HREI-MS to be $C_{31}H_{57}NO_8$. Its ^{1}H NMR spectrum was nearly identical to that of **1**. However, signals due to the propionyl group (δ 2.41 (2H, q, $J=7.5$ Hz) and 1.17 (3H, t, $J=7.5$ Hz)) of **1** disappeared in the case of **2**, while signals of two methyl groups (δ 0.98 (6H, d, $J=6.7$ Hz)), a methylene (δ 2.26 (2H, d, $J=7.0$)) and a methine (δ 2.07–2.15 (1H, m)), which composed the isovaleryl group, were instead observed. These findings revealed that the proionyloxy group in the C-6 position in compound **1** was replaced with an isovaleryloxy group in compound **2**.

To obtain sufficient samples for performing a biological evaluation, we carried out syntheses of **1** and **2** (Scheme



Scheme 1. *Reagent and conditions*: (a) 2% H_2SO_4, MeOH, rt (25%); (b) 2% H_2SO_4, MeOH, reflux; (c) Ac_2O, pyridine, rt (two steps, 25% from **1** and 38% from *N*-acetyl-D-glucosamine).



Scheme 2. *Reagent and conditions*: (a) allyl alcohol, *p*TsOH, reflux; (b) Ac_2O, pyridine, rt (two steps, 64%); (c) MeONa, MeOH, rt; (d) 2,2-dimethoxypropane, *p*TsOH, DMF, rt (two steps, 97%); (e) stearoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt (71%); (f) Dowex 50W (H^+), MeOH, rt (80%); (g) propionyl chloride, pyridine, 0°C (93% for **9a**) or isovaleric acid, EDCI, DMAP, CH_2Cl_2, 0°C (42% for **9b**); (h) $Rh(PPh_3)_3Cl$, DABCO, EtOH–H_2O (9:1), reflux; (i) microencapsulated OsO_4, NMO, H_2O–acetonitrile–acetone (1:1:1), rt (32% for **1** and 55% for **2**).

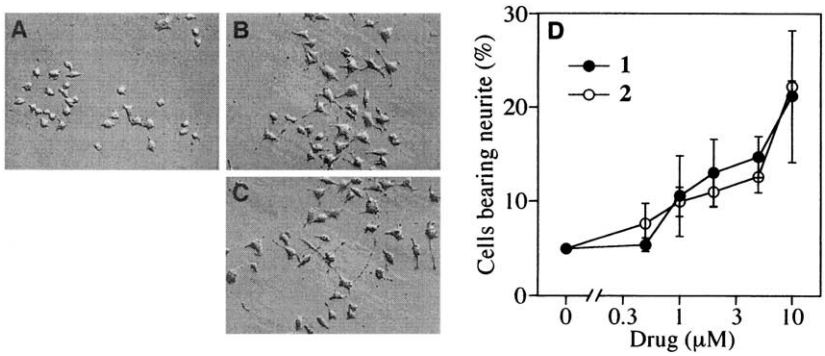


Figure 1. Effects of dictyoglucosamine A (**1**) and B (**2**) on morphological differentiation of PC-12 cells. PC-12 cells were cultivated in the absence (A) or presence of 10 μM of **1** (B) or **2** (C). The concentration-dependency of **1** or **2** is shown in D. Each point represents the mean with S.E. of three independent experiments.

2). Treatment of *N*-acetyl-D-glucosamine (**5**) as a starting material with *p*-toluenesulfonic acid in allyl alcohol gave a mixture of the allyl glycoside and its deacetylated product. Acetylation of the mixture with acetic anhydride in pyridine afforded a mixture of α- and β-anomers of the tetraacetylated compound. Deaceylation of this α-anomer (**6**), using sodium methoxide and subsequent acetonation, with 2,2-dimethoxypropane, gave the 4,6-isopropylidene derivative (**7**). After esterification of **7** with stearoyl chloride, deacetonation was performed under acidic conditions to obtain stearate **8**. Regioselective esterification of **8** with propionyl chloride in pyridine at 0°C gave propionate **9a**. The treatment of **9a** with Wilkinson's catalyst in 10% aqueous ethanol followed by oxidative cleavage of 1-propenyl glycoside with microencapsulated osmium tetroxide allowed us to complete the synthesis of **1**. Isovalarate **9b**, which was obtained from **8** with isovaleric acid in the presence of EDCI and DMAP, was converted into **2** in the same manner as in the synthesis of **1**. All of the spectral data of synthetic **1** and **2** were identical with those of natural compounds.

Biological evaluations of **1** and **2** on rat pheochromocytoma (PC-12) cells were performed. PC-12 cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 0.5% FCS with **1** or **2** at a concentration of 0.5 to 10 μM for 4 days, after which the neurite outgrowth of cells was observed under a phase-contrast microscope (Fig. 1). Compounds **1** and **2** caused the morphological change of PC-12 cells to extend neurites in a concentration-dependent manner.

It should be mentioned that these compounds are characteristic in the point that the amino sugar part is directly connected to the fatty acid. In addition to **1** and **2**, furanodictine A and B were also isolated from cellular slime molds. These compounds are known acylated amino sugar derivatives that possess neuronal differentiation activity.[4] Thus, a structure–activity relationship study of amino sugar analogues may lead to the discovery of novel nerve-rejuvenation drugs.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 12470474) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

1. Maeda, Y.; Inouye, K.; Takeuchi, I., Eds. *Dictyostelium A Model System for Cell and Developmental Biology*; Frontiers Science Series No. 21; Universal Academy Press: Tokyo, 1997.
2. Takaya, Y.; Kikuchi, H.; Terui, Y.; Komiya, J.; Furukawa, K.; Seya, K.; Motomura, S.; Ito, A.; Oshima, Y. *J. Org. Chem.* **2000**, *65*, 985–989.
3. Takaya, Y.; Kikuchi, H.; Terui, Y.; Komiya, J.; Maeda, Y.; Ito, A.; Oshima, Y. *Tetrahedron Lett.* **2001**, *42*, 61–63.
4. Kikuchi, H.; Saito, Y.; Komiya, J.; Takaya, Y.; Honma, S.; Nakahata, N.; Ito, A.; Oshima, Y. *J. Org. Chem.* **2001**, *66*, 6982–6987.
5. Maeda, Y.; Kikuchi, H.; Amagai, A.; Sasaki, K.; Sekiya, J.; Takaya, Y.; Oshima, Y. *FEBS Lett.*, submitted.
6. Data for **1**: colorless amorphous; $[\alpha]_D$ +11.9 (*c* 0.960, $CHCl_3$); IR (cm^{-1}) 3295, 2922, 2852, 1740, 1660. Data for **2**: colorless amorphous; $[\alpha]_D$ +18.2 (*c* 0.011, $CHCl_3$); IR (cm^{-1}) 3395, 2924, 2854, 1742, 1657. ^1H and ^{13}C NMR data of **1** and **2** are shown in Table 1.
7. Data for **3**: ^1H NMR (500 MHz, $CDCl_3$) δ 5.92 (1H, d, $J=9.6$ Hz, NH), 5.26 (1H, d, $J=3.4$ Hz, H-1), 5.18 (1H, dd, $J=9.6$, 9.1 Hz, H-3), 4.20 (1H, td, $J=9.6$, 3.4 Hz, H-2), 3.96 (1H, ddd, $J=9.1$, 4.0, 3.0 Hz, H-5), 3.89 (1H, dd, $J=12.1$, 3.0 Hz, H-6), 3.84 (1H, dd, $J=12.1$, 4.0 Hz, H-6), 3.76 (1H, t, $J=9.1$ Hz, H-4), 2.36 (1H, dt, $J=15.5$, 7.7 Hz, H-2″), 2.35 (1H, dt, $J=15.5$, 7.6 Hz, H-2″), 1.96 (3H, s, H-2′), 1.55–1.61 (2H, m, H-3″), 1.20–1.35 (28H, m, H-4″–H-17″), 0.88 (3H, t, $J=6.9$ Hz, H-18″); EI-MS *m*/*z* 469 $[M-H_2O]^+$, 284, 266, 55 (base); HREI-MS *m*/*z* 469.3415 (469.3400 calcd for $C_{26}H_{47}NO_6$); FAB-MS *m*/*z* 488 $[M+H]^+$.