Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 13, pp. 1619-1622, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00199-5

SYNTHESIS AND STRUCTURE - ANTI-TUMOR-PROMOTING ACTIVITY RELATIONSHIP OF MONOGALACTOSYL DIACYLGLYCEROLS

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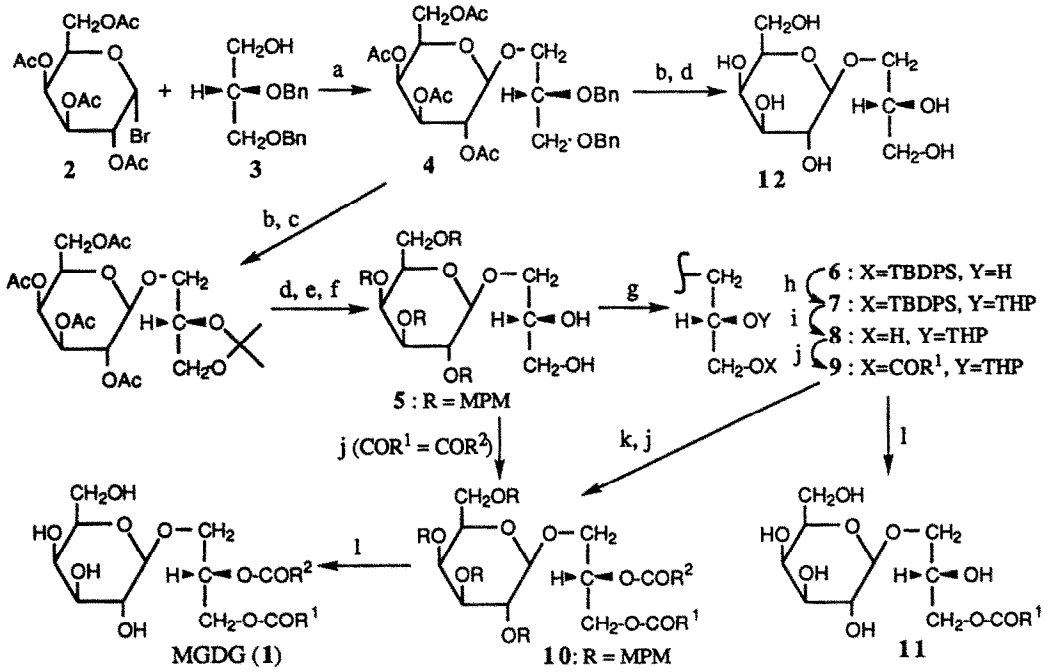
Abstract Monogalactosyl diacylglycerols (MGDG, **1a-t**) were synthesized utilizing *p*-methoxybenzyl group for protection of hydroxyl groups at sugar moiety. Inhibitory activity of MGDG (**1a-t**) and related compounds on Epstein-Barr virus early antigen activation was evaluated as anti-tumor-promoting activity, and MGDG with oleoyl group (**1c,h-j,p,s**) showed strong activity.

Glyceroglycolipids are major constituents of the chloroplast membrane in the plant kingdom and attract much attention in recent years because of their biological activities. Monogalactosyl diacylglycerols (MGDG, **1**), a class of glyceroglycolipids, were reported as antifauling substances,[3] a *Dacus cucurbitae* attractant,[4] and anti-inflammatory substances.[5] On our course of the investigation of biologically active compounds from fresh-water microalgae, we have also isolated 8 MGDG as anti-tumor-promoting substances as well as 9 digalactosyl diacylglycerols (DGDG).[6] Although some MGDG and DGDG were more potent than the others, no apparent relationship was observed between acyl pairs and the activity. Since it is difficult to get various MGDG as natural products, we attempted to synthesize MGDG with various acyl pairs to clarify the relationship. As saturated acyl groups of natural MGDG are mainly at *sn*-2 position and unsaturated ones are at *sn*-1 position in the case that they possess both types of acyl groups, we planned to prepare MGDG with saturated ones at *sn*-1 position and unsaturated ones at *sn*-2 position. We also planned to obtain MGDG with highly unsaturated acyl groups such as docosahexaenoyl and icosapentaenoyl groups which were supposed to prevent carcinogenesis.[7] We now report synthesis of MGDG (**1**) with desired acyl groups at desired positions and elucidation of their anti-tumor-promoting activity.

The synthetic route to MGDG (**1**) is shown in Scheme. Several examples of MGDG synthesis were reported,[8] and in most cases, the hydroxyl groups at sugar moiety were protected as acetyl or benzyl groups



Scheme : (a) HgO, $HgBr_2$, Drierite, $ClCH_2CH_2Cl$, r.t., 4 h; (b) H_2, 10% Pd/C, AcOEt-EtOH-AcOH, 5 atm, r.t., 2 d; (c) $(MeO)_2Me_2$, *p*-toluenesulfonic acid (TsOH), DMF, r.t., 1.5 h; (d) MeONa, MeOH, r.t., 10 min; (e) MPM-Cl, NaH, DMF, r.t., 5 h; (f) TsOH, MeOH, r.t., 2 h; (g) TBDPS-Cl, pyridine, r.t., 4 h; (h) dihydropyrane, pyridinium *p*-toluenesulfonate (PPTS), CH_2Cl_2, r.t., 3 h; (i) *n*-Bu_4NF, THF, r.t., 5 h; (j) fatty acid, dicyclohexylcarbodiimide, dimethylaminopyridine, CH_2Cl_2, r.t., 2 h; (k) PPTS, MeOH, r.t., 13 h; (l) CAN, MeCN-H_2O, r.t., 45 min

which were removed at the final steps under reductive conditions.[8b-e] At the conditions, MGDG with highly unsaturated acyl groups were obtained only in low yield.[9] Thus we used *p*-methoxybenzyl (MPM) group which can be easily cleaved under mild conditions. Dibenzyl-*sn*-glycerol (3) from D-mannitol[8b,10] was reacted with **2**[11] in the presence of HgO and $HgBr_2$ to give β anomer (4, 91%). After the benzyl groups of 4 were converted to an isopropylidene acetal group, the acetyl groups at sugar moiety were cleaved followed by protection of the resulting hydroxyl groups as MPM ethers.[12] Then the isopropylidene acetal group was removed to afford **5**[12](86% from 4). In order to introduce desired acyl groups at desired positions in good yield, the hydroxyl groups at *sn*-1 and -2 positions were protected individually as *t*-butyldiphenylsilyl (TBDPS) ether[12] and tetrahydropyranyl (THP) ether, respectively, to give **7**[12](84% from 5). The TBDPS ether was cleaved to **8**[12] (98%), then acyl groups was incorporated at *sn*-1 position to give corresponding **9**[12](86-95%). After removal of the THP ether of 9, corresponding acyl groups were condensed at *sn*-2 position to afford **10**[12,13] (77-98%). Finally, the MPM ethers of **10** were removed by treatment with ceric ammonium nitrate (CAN), and MGDG

Table Inhibitory Activity of Synthetic MGDG (**1a-t**) and Related Compounds (**11a-c** and **12**) on TPA-induced EBV-EA[a] Activation

Compounds	Acyl group		Concentration (mol ratio/TPA) 1000	500	100	10
	sn-1 (COR^1)	*sn*-2 (COR^2)	% to control ±S.E.[b]		(% viability)	
1a[c]	myristoyl	acetyl	17.4±2.2 (>70)	36.8±2.5	52.9±3.8	77.4±3.0
1b[c]	myristoyl	myristoyl (C14:0)	0 ±0.5 (>70)	15.4±1.9	88.2±2.0	100
1c[c]	myristoyl	oleoyl (C18:1)	0 ±0 (>70)	16.9±1.8	80.4±4.4	100
1d[c]	myristoyl	linoleoyl (C18:2)	17.1±5.0 (70)	39.6±4.4	88.2±1.0	100
1e[c]	myristoyl	α-linolenoyl (C18:3)	0 ±0.3 (80)	20.2±4.6	73.9±1.1	100
1f[c]	myristoyl	icosapentaenoyl (C20:5)	26.1±1.1 (>70)	52.8±3.6	84.1±1.8	100
1g[c]	myristoyl	docosahexaenoyl (C22:6)	0 ±1.5 (>60)	64.7±4.2	88.3±2.0	100
1h	oleoyl	oleoyl	0 ±0 (70)	11.0±2.0	21.3±1.8	61.0±0.5
1i[c]	oleoyl	icosapentaenoyl	0 ±0.3 (>70)	18.5±2.8	69.6±3.3	93.2±2.0
1j[c]	oleoyl	docosahexaenoyl	0 ±0.6 (>70)	26.4±1.8	68.1±3.2	88.4±3.1
1k	linoleoyl	palmitoleoyl (C16:1)	53.3±4.2 (70)	74.6±1.5	100	100
1l	linoleoyl	linoleoyl	14.7±4.4 (70)	35.8±2.3	79.0±1.3	100
1m	α-linolenoyl	palmitoyl (C16:0)	19.4±3.9 (80)	57.6±3.0	92.6±0.1	100
1n[c]	α-linolenoyl	linoleoyl	8.7±3.0 (70)	22.2±2.6	64.5±2.5	84.9±0.3
1o[c]	icosapentaenoyl	myristoyl	15.8±2.9 (80)	30.6±1.9	72.8±2.8	90.7±0.6
1p[c]	icosapentaenoyl	oleoyl	0 ±0 (>70)	0 ±0.5	53.6±3.9	81.7±3.5
1q[c]	icosapentaenoyl	icosapentaenoyl	0 ±0.4 (80)	54.3±3.0	89.0±1.7	100
1r[c]	docosahexaenoyl	myristoyl	13.8±2.2 (80)	29.7±2.9	68.9±2.6	100
1s[c]	docosahexaenoyl	oleoyl	0 ±0.3 (>70)	12.8±3.0	62.9±4.2	83.2±2.7
1t[c]	docosahexaenoyl	docosahexaenoyl	0 ±0 (80)	26.3±1.5	64.8±3.1	86.8±1.0
11a	myristoyl	H	0 ±0.6 (>70)	22.5±1.4	65.3±2.9	90.7±0.4
11b	oleoyl	H	0 ±0 (>70)	0 ±0.8	84.7±1.8	100
11c	linoleoyl	H	0 ±0.7 (>70)	24.1±2.0	79.8±3.5	100
12	H	H	0 ±0.2 (>70)	15.8±2.5	66.7±3.7	100

a) Epstein-Barr Virus Early Antigen. b) Standard error (n=3). c) New MGDG.[12]

with different acyl groups (**1a**,**c-g**,**i-k**,**m-p**,**r-s**) were obtained in moderate yield (26-42% from **3** for 13 steps). MGDG with the same acyl groups at *sn*-1 and -2 positions (**1b**,**h**,**l**,**q**,**t**) were obtained by acylation of **5** followed by cleavage of the MPM ethers. *Sn*-2 lyso derivatives (**11a-c**) were directly given from corresponding **9** by treatment with CAN. Galactosyl glycerol (**12**) was obtained by removal of the protective proups of **4**.

The anti-tumor-promoting activities of the compounds were determined using a short-term *in vitro* assay of Epstein-Barr virus activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as described before.[6,14] The activity of each compound is shown in Table. No samples exhibited cytotoxicity at the concentration of 1000 mol ratio / TPA. The synthetic MGDG with same acyl pair that we isolated from alga was checked its activity and indicated almost the same activity as the natural one. Considering of the activity of MGDG including natural ones at the 1000 mol ratio / TPA concentration,[15] MGDG with a myristoyl group at *sn*-1 position (**1a-g**) were more potent than those at *sn*-2 position. Although MGDG with highly unsaturated acyl group including **1o** and **1r**, which have myristoyl group at *sn*-2 position, showed relatively strong effect.

MGDG with oleoyl group (**1c,h-j,p,s**) also completely inhibited EBV-EA activation at the concentration of 1000 mol ratio per TPA. It is noteworthy that galactosyl glycerol (**12**) indicated the anti-tumor-promoting activity as well as *sn*-2 lyso derivatives (**11**). This fact suggests that **12** is the principal structure for appearance of the activity of MGDG, and the strength of the activity should depend on the acyl groups. Although most MGDG were less potent than **12**, only MGDG with oleoyl group (**1c,h-j,p,s**) exhibited almost the same or stronger activity than **12**. Dioleoyl derivative (**1h**) was the most potent in the MGDG we tested.

In summary, we established sufficient synthetic route to MGDG (**1**) with desired acyl groups at desired positions by use of MPM ether for protection of the hydroxyl groups at the sugar moiety, and obtained 20 kinds of MGDG including 16 new ones. As the result of the evaluation of their anti-tumor-promoting activity, we clarified that oleoyl group contributed the most to the activity of MGDG among various acyl groups we tested, and dioleoyl derivative (**1h**) indicated the strongest activity.

Acknowledgments. We thank Ms. S. Kato, Ms. Nakano and Ms. Takahashi of this Faculty for ^{1}H NMR and FAB MS measurements. This work was supported in part by Grant-in-Aid for Scientific Research from Ministry of Education, Science, and Culture of Japan. We are also grateful to Toyo Igaku Kenkyu Foundation for financial support.

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15. Inhibitory effects of the natural MGDG on TPA-induced EBV-EA activation at the concentration of 1000 mol ratio / TPA ^{6}: (acyl group at *sn*-1 position / *sn*-2 position : % to control); palmitoyl / myristoyl : 58.7, palmitoleoyl : 56.3, oleoyl / myristoyl : 62.2, linoleoyl / myristoyl : 51.4, α-linolenoyl / myristoyl : 67.8, linoleoyl / palmitoyl : 69.4, α-linolenoyl / palmitoleoyl : 60.7, α-linolenoyl / α-linolenoyl : 87.7.

(*Received in Japan* 28 *April* 1994; *accepted* 24 *May* 1994)