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Mendeleev Commun., 2015, **25**, 248–249
Mendeleev Communications

Synthesis and antitumor activity of new alkyl glycoglycerolipids

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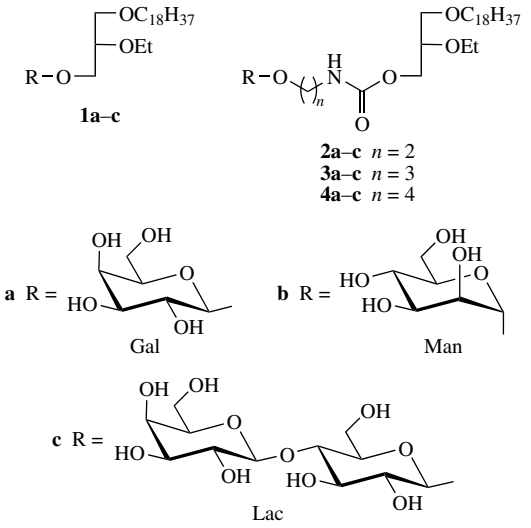
DOI: 10.1016/j.mencom.2015.07.003

New glycosylated alkyl glycerolipids possessing antitumour activity were synthesized from 2-*O*-ethyl-1-*O*-octadecylglycerol, amino alcohols and monosaccharides.

Alkyl glycerolipids (antitumour ether lipids, AELs) exhibit antitumour activity.[1,2] The prototype compound, edelfosine (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine, ET-18-OMe), kills tumour cells and shows no mutagenic effects,[3–5] however, its side effect, hemolysis, limits the use of this compound.[6] Therefore, search for antitumour agents in chemical classes with similar structures, including non-phosphorus glycoglycerolipids[1,7–9] is topical.

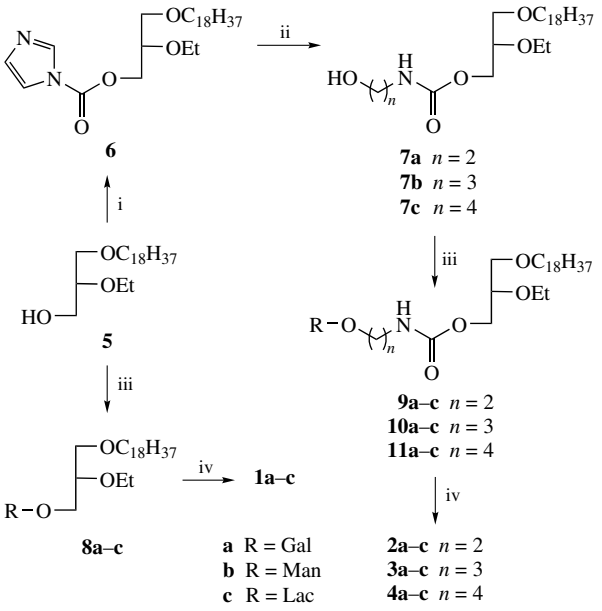
Carbohydrate derivatives of antitumour ether glycerolipids (glycosylated antitumor ether lipids, GAELs) demonstrate an antiproliferative effect[10–12] and inhibit migration, invasion and metastasis.[13–15] Preferential toxicity to tumour cells, lack of mutagenicity, beneficial physicochemical and pharmacological properties (small molecule size, amphiphilicity, stability in storage, suitability for intravenous and oral administration) indicate that GAELs can be used both separately and in combinations with other compounds and therapeutic methods.[10]

GAELs differ in the nature of carbohydrate residues, type of glycoside bond and its anomeric configuration.[10,11,16–18] The lipophilic part of GAEL structures is represented by enantiomers that contain a chiral centre at the C-2 position of the glycerol skeleton which is usually bound to the carbohydrate by O-, S- or C-glycosidic bonds. Synthesis of such compounds involves multiple stages and requires conditions preventing racemization. In the present work we have synthesized new glycolipids with a racemic diglyceride backbone, in which the lipid part is connected to the carbohydrate directly (**1a–c**) or through a spacer group (**2–4**). We have studied the capability of the new compounds to kill tumour cells and to lyse erythrocytes.



All the new GAELs have a common structural moiety, namely, racemic 2-*O*-ethyl-1-*O*-octadecyl-*rac*-glycerol, that we previously used in a synthesis of antitumor phosphor-free AELs.[8,9] D-Galactose, D-mannose and lactose residues were used as the carbohydrate components. Furthermore, varying the distance between the carbohydrate residues and the diglyceride backbone allowed us to evaluate the effect of the spacer group on the antitumour properties of GAELs.

The synthesis of new GAELs involved the Helferich glycosylation[19] in the presence of $HgBr_2$ and $Hg(CN)_2$ as the key stage (Scheme 1). In the case of GAELs **1a–c**, the starting diglyceride **5** was subjected to glycosylation. To obtain spacer-containing glycolipids **2–4**, spacer groups were attached to molecule **5** by creating a carbamoyl bond. For this purpose, compound **5** was treated with a threefold excess of carbonyldiimidazole in the presence of triethylamine to give imidazolide **6**. Subsequent reactions of activated diglyceride **6** with amino alcohols (2-aminoethanol, 3-aminopropanol, 4-aminobutanol) afforded compounds **7a–c**. The reaction time was 10–19 h, depending on the amino alcohol structure. Products **7a–c** were isolated by column chromatography on silica gel in 68–89% yields.



Scheme 1 *Reagents and conditions*: i, CDI, TEA, DCM, 50 °C, 10 h; ii, $H_2N(CH_2)_nOH$ (n = 2–4), DCM, 50 °C, 10 h; iii, $Hg(CN)_2$, $HgBr_2$, 4 Å molecular sieves, DCM, 20 °C, 9–24 h; iv, 0.1 N MeONa/MeOH, $CHCl_3$, 20 °C, 15–60 min.

The best yields of glycosides **8** (45–79%) and **9–11** (40–71%)† were achieved in reactions of compounds **5** and **7a–c** with a threefold excess of glycosyl bromides at room temperature in dichloromethane. The glycosylation time providing the most complete conversion of the initial diglyceride substrate was 9–24 h. Compounds **8–11** were characterized by mass spectrometric data as well as ^{1}H and ^{13}C NMR data.† The ^{1}H NMR spectra of glycosides **8a**,**c**–**11a**,**c** revealed proton signals at anomeric centers as doublets with a chemical shift of δ 4.39–4.46 ppm and spin–spin coupling constants $J_{1,2}$ 7.7–7.9 Hz, which correspond to the axial position of anomeric protons in D-galactose and lactose. The signal of the anomeric proton with the constant $J_{1,2}$ 1.6 Hz for α-mannosides **8b–11b** was observed at δ 4.73 ppm. The signals of anomeric carbon atoms in β-glycosides **8a**,**c**–**11a**,**c** in the ^{13}C NMR spectra were located at δ 101.38–101.55 ppm, while those for α-mannosides **8b–11b** were located at δ 97.28–97.70 ppm. Removal of protecting acetyl groups in compounds **8–11** by treatment with 0.1 N sodium methoxide solution in methanol gave the target GAELs **1–4** (yields 68–93%). The structures of these compounds were confirmed by MS and NMR methods.†

We studied the ability of the new GAELs **1–4** to induce mammalian cancer cells death and to cause hemolysis. The cytotoxicity of the compounds synthesized was estimated using a colorimetric test[20] by determining concentrations that caused the death of 50% tumor cells (IC_{50}) (Table 1).

In certain cell lines, GAELs with D-galactose (**1a**) and D-mannose residues (**1b**, **3b**) showed activity in the micromolar concentration range. Glycosylated glycerolipids **3a–c** with trimethylene spacer are the most active among the spacer-containing GAELs. It is important that compounds **1a**,**b** caused only moderate cytotoxicity for non-tumour fibroblasts: 70–80% cells survived even at 50 μM **1a** or **1b**. Lipid **1b** with D-mannose residue was least toxic for fibroblasts. Edelfosine was found to be somewhat more toxic (IC_{50} ~ 50 μM) for skin fibroblasts (see Online Supplementary Materials).

Hemolysis (violation of erythrocyte integrity) is an unfavourable side effect of edelfosine.[6,21] Comparison of the ability to damage erythrocytes showed that the new GAELs **1a**,**b** (5–20 μM) destroyed only ~3% erythrocytes, whereas hemolysis by equitoxic concentrations of edelfosine was considerably stronger (Figure 1).

In conclusion, we have synthesized new GAELs with carbohydrate residues bound to a diglyceride directly or through a spacer group. A study of their cytotoxicity showed that high activity was manifested by the GAELs in which D-galactose and D-mannose residues were linked to the glycerol skeleton either directly or through a trimethylene spacer. At concentrations that kill tumour cells, these compounds possess low toxicity toward non-tumour cells and do not damage erythrocytes, which is an advantage of the new GAELs over edelfosine. The results presented here confirm that further development of the new class of glycosylated glycerolipids as antitumour agents is expedient.

Table 1 Cytotoxicity of GAELs **1–4** for tumour cell lines.[a]

Compound	IC_{50}/μM			
	K562	HCT116	B16	HL60
Edelfosine	>50	n.d.[b]	6.0±1.1	n.d.
1a	2.5±0.9	n.d.	13.5±0.8	n.d.
1b	3.0±0.9	15.0±0.4	12.0±0.7	16.0±0.7
1c	18.0±0.7	42.0±0.6	>50	>50
2a	35.0±0.5	14.0±0.9	16.0±0.5	17.0±0.8
3a	17.0±0.5	19.0±0.4	n.d.	17.0±0.2
4a	29.0±0.2	n.d.	34.0±1.0	n.d.
2b	34.0±0.4	26.0±0.2	n.d.	29.0±0.4
3b	18.0±0.6	8.0±0.7	8.0±0.6	15.0±0.5
4b	36.0±0.6	39.0±0.2	n.d.	n.d.
2c	18.0±0.2	34.0±0.2	20.0±0.3	38.0±0.2
3c	7.0±0.5	18.0±0.4	15.0±0.5	18.0±0.4
4c	16.0±0.2	47.0±0.5	45.0±0.2	37.0±0.3

[a] The mean values and standard deviations based on three independent measurements are listed. [b] n.d. – not determined.

† See Online Supplementary Materials.

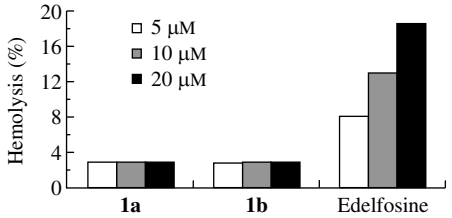


Figure 1 Hemolysis on exposure to equitoxic concentrations of **1a**,**b** and edelfosine. Mean values from 4 experiments are given. The value of 100% corresponds to hemolysis caused by lysis of erythrocytes with distilled water.

This study was carried out within a state assignment from the Ministry of Education and Science of the Russian Federation (project no. 4.128.2014/K).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.07.003.

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Received: 18th November 2014; Com. 14/4505