Anticancer Lead Discovery

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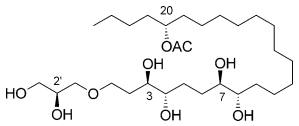
Mycalol: A Natural Lipid with Promising Cytotoxic Properties against Human Anaplastic Thyroid Carcinoma Cells**

Adele Cutignano, Genoveffa Nuzzo, Daniela D'Angelo, Eleonora Borbone, Alfredo Fusco, and Angelo Fontana**

The high-mobility group A (HMGA, types 1 and 2) proteins are low-molecular-weight nuclear factors that orchestrate the assembly of nucleoprotein complexes involved in gene transcription, replication, and chromatin structure. HMGAs possess oncogenic activity[1,2] and proteins of type 1 (HMGA1) have been correlated to cellular invasiveness and drug-resistance in human malignancies.[3] In particular, blockage of expression of these proteins significantly enhances the responsiveness of tumor cell lines that are otherwise resistant to cytotoxic agents. Thus, phenotypic assays based on cells with reduced levels of HMGA are a possible tool for a rational search of novel compounds against tumors whose aggressiveness and resistance reduce the success of normal screening methods.

Herein, we report the elucidation of the structure of mycalol (**1**), a novel polyoxygenated ether lipid that showed a promising in vitro specific activity against different cell lines derived from human anaplastic thyroid carcinoma (ATC), the most aggressive human thyroid gland malignancy.[4] Mycalol was identified by a novel screening method based on the parallel use of FRO cells, which are human ATC-derived cells with high constitutive levels of HMAG1, but not HMGA2, and FRO-asHMGA1 cells, a genetically modified population of FRO cells that stably express an anti-HMGA1 antisense construct that blocks HMGA1 synthesis.[5] The effects of extracts and fractions were measured on the paired cell lines by MTS proliferation assay.

Mycalol was isolated from a chloroform extract of the sponge *Mycale (Oxymycale) acerata Kirkpatrick 1907* collected along the coasts of Terra Nova Bay (Antarctica) during the Austral summer of 2005. The sponge, frozen soon after collection, was extracted with MeOH and fractionated according to a modified Kupchan method.[6] The chloroform extract showed no activity against FRO cells up to 50 $\mu g\,mL^{-1}$, but gave a good response ($IC_{50} = 7.5\ \mu g\,mL^{-1}$) against HMGA1-silenced FRO cells (FRO-asHMGA), thus supporting the potential of a novel screening method based on HMGA-interference. Sequential steps of silica gel radial chromatography and reverse-phase HPLC (see the Supporting information) gave alkyl glyceryl ether **1** together with a number of minor compounds that are still under study.



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The HR-ESI$^+$ MS spectrum of **1** showed a $M-Na^+$ ion at *m/z* 573.3959, thus accounting for the molecular formula $C_{29}H_{58}O_9$ (calcd *m/z* 573.3979 for $C_{29}H_{58}O_9Na$) and requiring only one formal unsaturation. Accordingly, ^1H and ^{13}C 2D NMR data indicated a C_{27} linear structure with nine oxygenated carbons and an acetyl group (for full assignment, see the Supporting Information, Table S1). COSY spectra identified seven of these carbons as part of a glycerol moiety (H1′ δ 3.85 and 3.90; H2′ δ 4.35; H3′ δ 4.08) and two *gem*-diol spin systems. On the other hand, the acetyl residue at C20 was inferred on the basis of a long-range TOCSY correlation of the down-shifted oxymethine proton at δ 5.07 with the terminal methyl at δ 0.82 through three methylene groups at δ 1.20 ($H_2$23), δ 1.33 ($H_2$22) and δ 1.56 ($H_2$21). A diagnostic HMBC correlation of C1′ of glycerol (73.8 ppm) with the methylene signals at δ 3.97 allowed us to join the glyceryl residue with the linear chain through an ether linkage and to assign the last oxygenated function to C1.

This methylene group showed scalar couplings with the diastereomeric protons at δ 2.40 and δ 2.16 ($H_2$2), both also coupled to one of the oxymethine protons (δ 4.18, H3) of the two *gem*-diol systems. Characterization and location of these groups were unambiguously accomplished by 2D NMR analysis of the triacetonide derivatives **2** and **3** obtained by treatment of **1** with deuterated acetone and dimethoxypropane, respectively (Figure 1a). In particular, the deuterated derivative **2** allowed an easier inspection of the up-field region in 2D NMR experiments (see the Supporting Information), which revealed two methylene groups at δ 1.57 ($H_2$5) and 1.74 ($H_2$6) between the two diol moieties. The relative

[*] Dr. A. Cutignano, Dr. G. Nuzzo, Dr. A. Fontana
Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, 80078, Pozzuoli, Napoli (Italy)
E-mail: afontana@icb.cnr.it
Homepage: http://www.icb.cnr.it
Dr. D. D'Angelo, Dr. E. Borbone, Dr. A. Fusco
Istituto di Endocrinologia ed Oncologia Sperimentale, Consiglio Nazionale delle Ricerche, Via Pansini 5, 80131, Napoli (Italy)

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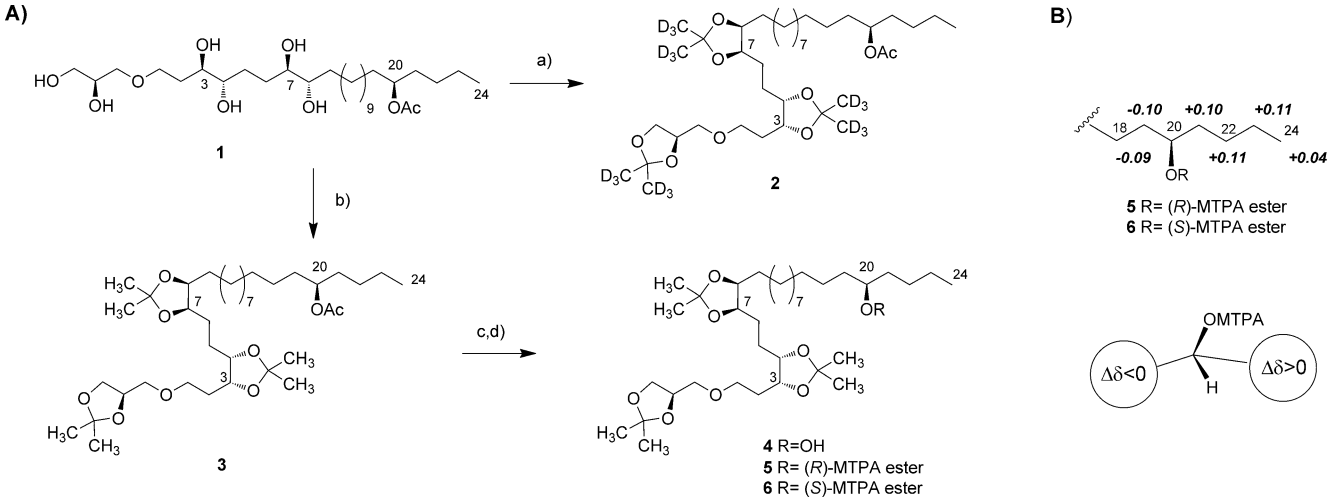
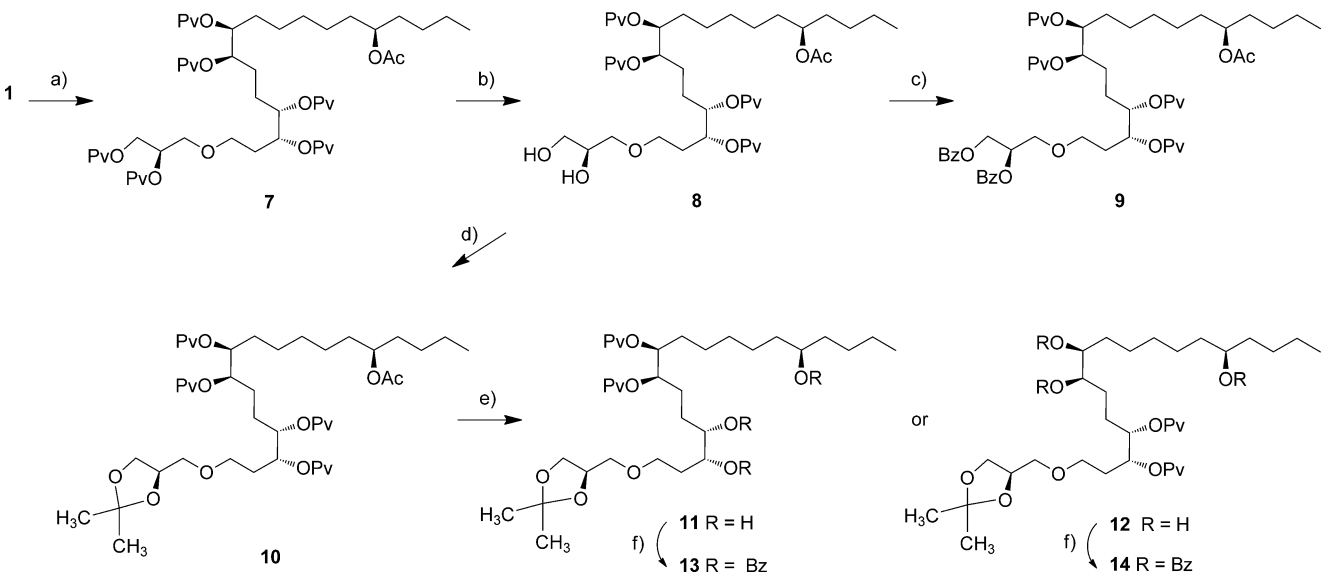


Figure 1. A) Synthesis of acetonide and MTPA derivatives of mycalol (**1**). B) $\Delta\delta$ ($\delta_S-\delta_R$) values (ppm) for selected protons of (*R*)-MTPA ester **5** and (*S*)-MTPA ester **6**. Reagents and conditions: a) $(CD_3)_2CO$, I_2, RT, 24 h; b) $(CH_3)_2C(OCH_3)_2$, PPTS, 75 °C, 6 h; c) Na_2CO_3, MeOH, 55 °C, 28 h; d) *S*-(−)- or *R*-(−)-MTPA chloride, DMAP, dry CH_2Cl_2, RT, overnight. Ac = acetyl, DMAP = 4-dimethylaminopyridine, MTPA = Mosher's acid; α-methoxy-α-(trifluoromethyl)phenylacetic acid, PPTS = pyridinium *p*-toluenesulfonate.

stereochemistry of these latter functions was established by the acetonide method on derivative **3**,[7] whose NMR spectrum in $CDCl_3$ showed well-separated methyl acetals (27.1 and 25.9 ppm; 26.8 and 25.4 ppm), which is in agreement with the *erythro* configuration for both *vic* diols of **1** (see the Supporting Information). The depicted *erythro* configuration was also supported by the small coupling constants that could be estimated between the vicinal protons in the dioxolane rings.

After methanolysis to generate alcohol **4** (Figure 1), acetonide **3** was also used to determine the absolute stereochemistry of the isolated hydroxy group at C20 by a modification of Mosher's method.[8] Despite the near symmetry around this stereocenter, 2D NMR analysis of MTPA derivatives **5** and **6** clearly indicated $\Delta\delta$ values in agreement with an *R* configuration for this carbon center (see the Supporting Information). On the other hand, the absolute stereochemistry of the central carbon of the substituted glycerol was deduced by the dibenzoate chirality method[9] on derivative **9**, which was obtained from the perpivaloyl derivative **7** by mild basic hydrolysis (Scheme 1). After purification by reverse-phase HPLC, compound **9** showed



Scheme 1. Selective derivatization of mycalol (**1**). a) PvCl, dry pyridine, RT; b) Na_2CO_3, MeOH RT, overnight; c) BzCl (4 equiv), dry pyridine, RT overnight; d) $(CH_3)_2C(OCH_3)_2$, PPTS, 75 °C, 6 h; e) Na_2CO_3, MeOH RT, 72 h, chromatographic resolution; f) excess BzCl, dry pyridine, 50 °C, 24 h. Bz = benzoyl, Pv = pivaloyl.

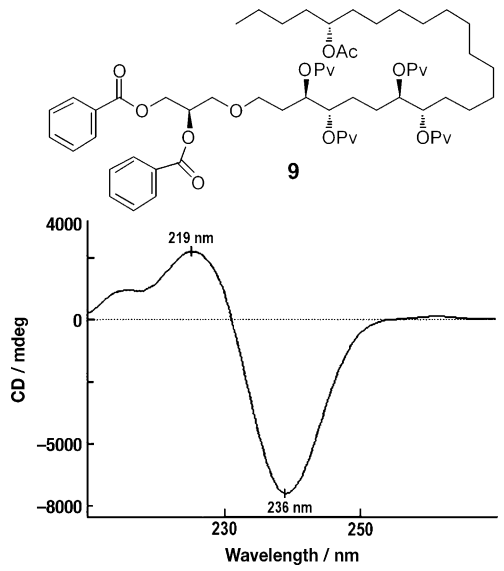


Figure 2. Circular dichroism curve of 1′,2′-dibenzoyl-3,4,7,8-tetrapivaloyl mycalol (**9**).

a CD spectrum with a negative first Cotton effect (CE; 236 nm) and a positive second CE (216 nm; Figure 2). Sign and wavelength of the Davydov splitting of **9** were identical to those reported for mono-1-*O*-alkyl *sn*-glycerol,[10] thus defining as *S* the absolute configuration at C2′ of natural product **1**.

Determination of the absolute configuration of the two *erythro* diol systems was also addressed by the chiroptical approach. In consideration of the difficulties in assigning the stereochemistry of *erythro* diols in flexible compounds,[7c] we first used an approach based on auxiliary chomophore dimolybdenum tetraacetate ($[Mo_2(AcO)_4]$), in accordance with the revised method developed by Snatzke and Frelek,[11a] which was further confirmed by the dibenzoate technique of Harada and Nakanishi.[9] The application of both methods required selective protection of the two *vic*-diol moieties and restriction of the conformational changes of the flexible chain of mycalol. After several attempts with different orthogonal protecting groups, both tasks were achieved by pivaloylation of the hydroxy groups followed by partial hydrolysis and chromatographic resolution of the two isomeric triols **11** and **12** (Scheme 1). The steric bulk of the pivaloyl groups significantly increased the rotational energy barriers of the linear chain in both compounds (see the Supporting Information). Therefore, in situ complexation with $[Mo_2(AcO)_4]$ induced a conformational preference that, in analogy with *erythro* and *threo* diol models reported in the literature,[11] produced CD spectra with four major bands in the range of 500–250 nm (Figure 3). The signs of the CEs centered around 300 nm (band IV) and 400 nm (band II), as well as a minimum or a maximum between these bands in **11** and **12**, respectively, were in full agreement with the assignment of 3*R*,4*S* and 7*R*,8*S* stereochemistry for the two diol moieties of **1** when the molecules adopt conformations with the part containing the pivaloyl groups antiperiplanar to the vicinal hydroxy group (Figure 3). This interpretation of the induced CD spectra of **11** and **12** is mostly dependent on the orientation of the

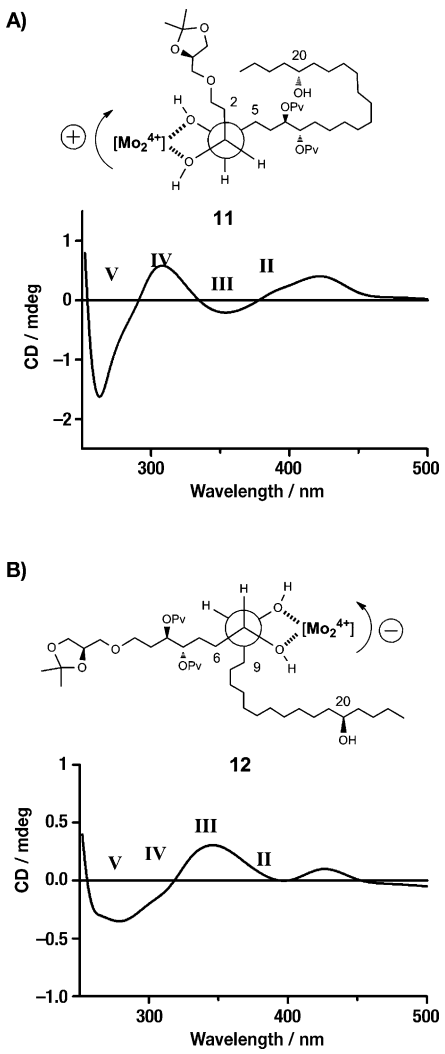


Figure 3. Circular dichroism curves of chiral MO_2–dimer complexes of the isolated diol derivatives **11** (A) and **12** (B). Roman numbers indicate the diagnostic CE bands following Snatzke and Frelek's nomenclature.

pivaloate-bearing substituents (see the Supporting Information). Whereas it is intuitive that the C5–C24 chain is larger than the dioxolane part for triol **11**, the differences between the two alkyl substituents around the C7/C8 diol in compound **12** is less obvious. To confirm that the conformational preferences are governed by the pivaloyl residues, and to support the configurational assignment of the *erythro-vic* diols in mycalol, compounds **11** and **12** were converted into benzoate derivatives **13** and **14** (Scheme 1). The resulting CD spectra (Figure 4) showed Cotton effects whose Davydov splitting, signs, and frequencies fully confirmed the 3*R*,4*S* and 7*R*,8*S* stereochemistry of the two diol systems.[9] Notably, the CD curve of compound **14** only agrees with the helicity rule described by Harada and Nakanishi for dibenzoate in the conformer where the substituent at C3 is *anti* to the benzoate group at C4 (see the Supporting Information), thus supporting the prediction about the conformational model proposed for complexes of **11** and **12** with $[Mo_2(AcO)_4]$.

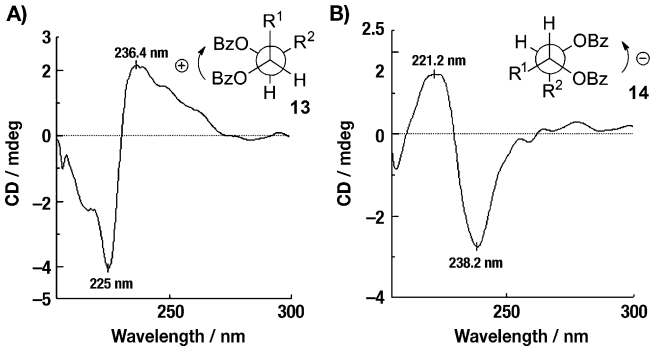


Figure 4. Circular dichroism curves of dibenzoyl derivatives **13** (A) and **14** (B). The inserts show the suggested chiralities of the two dibenzoates in agreement with Harada and Nakanishi model.[9] A, B) R^1 = glycerol-containing part. A) $R^2 = C_5$–C_{24} alkyl chain. B) $R^2 = C_9$–C_{24} alkyl chain.

Anaplastic thyroid carcinoma (ATC) causes up to 40% of all deaths from thyroid cancer.[4] The present chemotherapeutic treatments are mostly palliative, and currently there is no clinical candidate for the treatment of this disease. Mycalol exhibited considerable cytotoxicity on three ATC cell lines, with the highest effect being on the 8505c and ACT1 cells (IC_{50} 3.8 μM and 4.5 μM, respectively; Table 1). Notably, no

Table 1: Cytotoxic activity of mycalol (**1**).

Cell line		Source	IC_{50} [μM]
Human ATC-derived cell lines			
1	FRO	human ATC-derived cells	15.7
2	FRO-HMGA1as	FRO cells silenced for HMGA1	7.3
3	ACT1	human ATC-derived cells	4.5
4	8505c	human ATC-derived cells	3.8
Other solid tumor cell lines			
5	GEO	human colon cancer cells	n.a.[c]
6	GEO+HMGA1	GEO cells over-expressing HMGA1	n.a.[d]
7	HCT116	human colon carcinoma cells	10.9
8	OVCAR8	human ovarian cancer cells	n.a.[e]
9	MCF7	human breast adenocarcinoma cells	n.a.[f]
Functional thyroid cells			
10	FRTL5	rat thyroid cells	>36

[a] 95% cell vitality at 36 μM; [b] 85% cell vitality at 36 μM; [c] 81% cell vitality at 36 μM; [d] 76% cell vitality at 36 μM; [e] 92% cell vitality at 11 μM; [f] 72% cell vitality at 11 μM. n.a. = not active.

significant cytotoxicity was reported against differentiated rat thyrocytes (FRTL5 cells). Furthermore, except for a weak effect on colon carcinoma (IC50 10.9 μM), **1** did not affect the vitality of cell lines derived from other solid tumors.

Overall, these results indicate that the response orchestrated by HMGA1 proteins provides a simple and versatile in vitro tool for searching for novel agents for the treatment of this thyroid neoplasm. In this line of reasoning, it is noteworthy that the absence of an effect on thyrocytes transfected with activated ras oncogene (TL5-ky and TL5-ki; data not shown) or HMGA1-overexpressing colon carcinoma cells (GEO+HMGA1; Table 1) may be the result of the specific interference of **1** with the mechanisms leading to proliferation of ATC cells. Alkylglycerols are a known class of bioactive natural products with several biomedical and biochemical properties.[12] Nevertheless, to the best of our knowledge, **1** is the first member of the family to show such a wide extent of oxidation.[13]

In conclusion, we have identified a new polyoxygenated monoalkyl glyceryl ether, mycalol (**1**), with promising activity against ATC-derived cell lines. Full determination of the structure was deduced by a combination of chemical and chiroptical methods applied at the micromolar scale. Particularly relevant is the characterization of the two *erythro-vic* diol moieties by Snatzke and Frelek's method on conformationally constrained pivaloyl derivatives of **1**, which could open the possible application of this approach to other natural products with flexible structure. According to recent evidence on the cytotoxic effect of ecteinascidin-743 through impairment of the HMGA functions,[14] the assay based on HMGA1-interefered cells may result in an important method for screening of novel chemotherapeutic agents.

Experimental Section

General procedures: Optical rotations were measured on a Jasco DIP-370 digital polarimeter. CD spectra were acquired on a Jasco J-815 polarimeter. NMR spectra were recorded on a Bruker Avance DRX 600 equipped with a cryoprobe operating at 600 MHz for proton or a Bruker DRX 400 operating at 400 MHz for proton. Chemical shifts values are reported in ppm (δ) and referenced to internal signals of residual protons [C_5D_5N ^1H δ 7.19, ^{13}C 123.5 ppm; $CDCl_3$ ^1H δ7.26, ^{13}C 77.0 ppm; C_6D_6 ^1H δ7.15, ^{13}C 128.0 ppm). Mass spectra were acquired on a Q-Tof-*micro* mass spectrometer (Waters) equipped with an ESI source and a Lock-Spray apparatus for accurate mass measurements. Solvents were distilled prior to use.

Cell viability assay: Cells were exposed to extracts, fractions, or pure mycalol dissolved in DMSO. After 72 h, CellTiter 96 Aqueous One Solution (20 μL; Promega Italia, Milano, Italy) was dispensed, and the absorbance at 490 nm measured. The results were normalized for absorbance in the absence of compounds. Stable clones of HMGA1-Interfered FRO cells were prepared according to Ref. [5] by co-transfection of the plasmid together with pBabe-Puro vector containing puromycin resistance gene.

Mycalol (**1**): Colorless oil. HR-ESIMS$^+$ *m/z* 573.3959 $[M+Na]^+$ (calcd for $C_{29}H_{58}O_9Na$, 573.3979). $[\alpha]_D^{20} = +3.45$ ($c = 0.1$, MeOH); for ^1H and ^{13}C NMR data, see Table S1. Further experimental details can be found in the Supporting Information.

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