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Cytotoxicity effects of transition-metal chelators of the 5-substituted 2-hydroxyacetophenones and their oximes

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Summary — A series of 5-substituted 2-hydroxyacetophenones (**A**) and their oxime derivatives (**B**) were synthesized, and their cytotoxic activities towards MCF-7 breast cancer cells and B-16 melanoma cells were compared. The substitution on the benzylic position enabled variation of the lipophilic nature of the compound, while the chelating site remained free for metal binding. The results obtained indicate that the lipophilic properties of the compounds are favorable for cytotoxicity activity and that the oximes which are lipophilic enough (*eg*, **B-4**, **B-5**, **B-9**) are more potent than the corresponding ketones (**A-4**, **A-5**, **A-9**). All the potent oximes examined in this work showed strong cardiotoxicity.

chelator / oxime / cytotoxicity / cancer / melanoma / breast

Introduction

In the course of our studies on the cytotoxicity of various molecules incorporating sulfur-type donors (such as dimethylsulfoxide (DMSO) [1] and dimethylthiourea (DMTU) [2]), a question was raised about the common metal-chelating properties of those ligands. We proposed that control experiments performed in the presence of very well-known chelators would reveal the answer to this question. Then, in experiments with DMTU, various metal chelators (for alkali metal ions, transition metal ions and noble metal ions) were added (Nordenburg *et al*, unpublished results) and it was discovered that the DMTU solutions containing transition metal chelators as 8-hydroxyquinoline and hydroxyoximes [3], showed much greater cytotoxicity than the non-spiked DMTU solutions. Thus the strong cytotoxicity of transition metal chelators was revealed. A preliminary account of the common chelator 8-hydroxyquinoline [8-Hq] was described recently [4].

In order to develop a lead for a possible drug, we have chosen a very well-known class of transition metal chelators, α-hydroxyoximes, with high affinity for Cu(II), Fe(II) and Fe(III), and synthesized substituted hydroxyoximes, based on the following considerations:

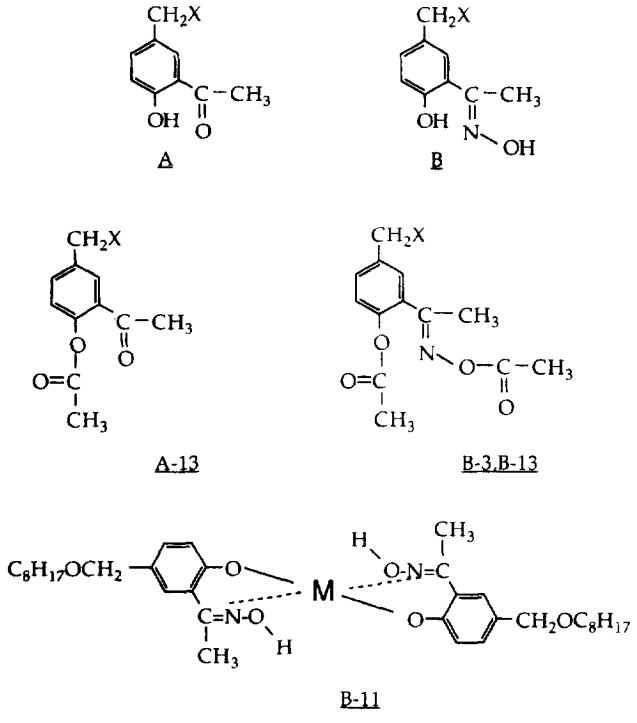
1. The chelating α-hydroxyoxime should be remote from the substituent and remain free for metal binding.
2. The substituent X should be varied to provide the molecule possible variance in lipophilic properties.

The combination of 1 and 2 allows us to determine structure–activity relationship and will provide insight into the preferred substituent for further development of a drug candidate.

Results and discussion

In the present study, the following questions were addressed: Is the cytotoxicity related to the metal-chelating strength of the chelator (ligand)? Is the cytotoxicity influenced by the overall hydrophilic/lipophilic nature of the chelator?

In order to answer the first question we have compared the activity of 5-substituted 2-hydroxyacetophenones (**A**) and their oxime derivatives (**B**), since it is known that the oximes are 4–5 orders of magnitude stronger ligands than the corresponding ketones [5]. We then blocked both the phenol and the oxime by an ester group (**A-13**, **B-3**, **B-13**) (the remaining =N-OR ligand is much weaker with the phenol blocked), or blocked the whole ligand site as a



metal complex with iron, copper or zinc **(B-11)**.

The answer to the second question was obtained by retaining the metal-chelating sites and varying the X substituents in the *para* position [6]. Variation of the X substituent will generally cause a strong effect on the hydrophobicity of the molecule, without a significant effect on the metal complexation ability.

Table I shows the structure of the 5-substituted 2-hydroxyacetophenones (class **A**) and their oximes (class **B**). Substitution on the benzylic position allows the synthesis of compounds with variable hydrophilic/lipophilic balance. The following types of 5-substituted chelators were synthesized: (a) neutral ethers, esters and carbinols (compounds **1–6**); (b) basic amines (compounds **7** and **9**); (c) positively charged quaternary amines (compounds **8**, **10** and **12**); (d) acetate-blocked phenols (compounds **3** and **13**); and (e) metal-blocked (compounds **11** (M = Fe, Zn, Cu)).

The synthesis of the compounds is presented in scheme 1 and consists of nucleophilic displacements of the chlorine atom in 3,4-disubstituted benzyl chlorides in non-aqueous solvents, as discussed in earlier publications [7, 8]. The resulting 5-substituted 2-hydroxyacetophenones are then oximated by hydroxylamine hydrochloride to the corresponding oximes (**B-2**, **B-4**, **B-5**, **B-7** and **B-9**). The acetylation of the acetylphenol **A-9** and the oximes **B-2** and **B-9** by acetic anhydride yields the acetate-blocked phenols **A-13**, **B-3** and **B-13**, respectively.

Complexation of the oxime **B-4** with Fe^{2+}, Cu^{2+}, Zn^{2+} and Fe^{3+}, gives the metal-blocked oxime **B-11**. Compounds **A-1** and **A-6** were synthesized by silica-catalyzed hydrolysis (**A-1**) or hydrolysis and condensation (**A-6**) (scheme 2).

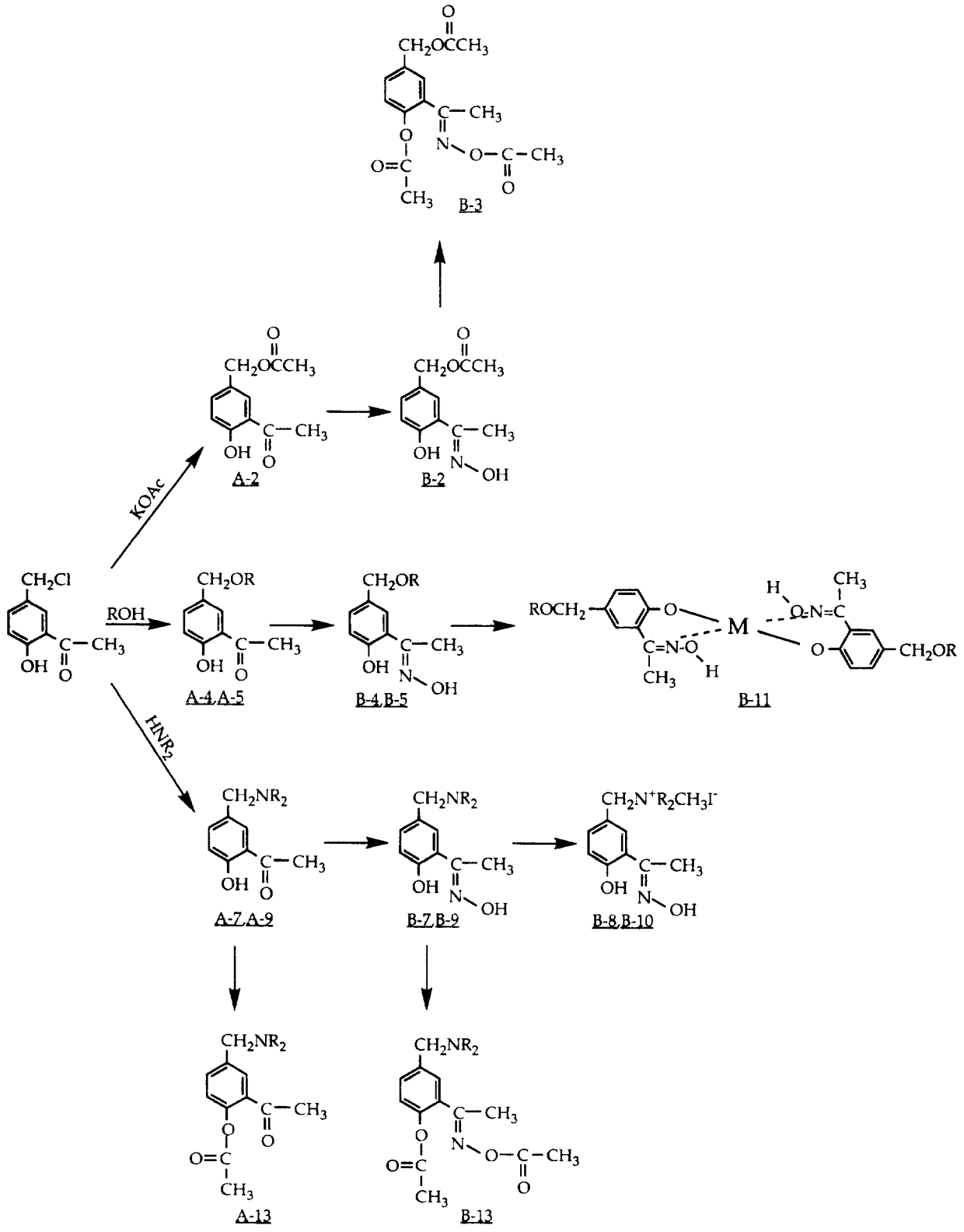
The activities of pairs of ketones and oximes towards **B-16** melanoma cells inhibition were compared (see table I) in an attempt to estimate the role of the metal-chelating strength of the chelator and the hydrophobic factor. From the results presented in table I we selected three pairs of compounds which exhibited a good inhibition effect on **B-16** melanoma cells (**4**, X = OC_8H_{17}; **5**, X = $[O(CH_2)_2]_2OC_4H_9$; and **9**, X = $N(C_8H_{17})_2$) and evaluated their action towards cell inhibition growth of MCF-7 breast cancer cells (table II).

From the results summarized in tables I and II it is possible to conclude that compounds with small X substituents and a hydrophilic nature (for example, **B-1**, **A-2**, **B-2** and **B-3**) show a poor inhibition effect while those with large X substituents and hydrophobic properties (for example, **B-4**, **B-5**, **A-9**, **B-9**, **A-13** and **B-13**) show a large inhibition effect. The activity of both ketones and oximes is concentration dependent, while the oximes are generally more potent than the ketones.

Compounds **B-4** (X = OC_8H_{17}, Y = H) and **B-9** (X = $N(C_8H_{17})_2$, Y = H) are the most effective chelators for inhibition of cancer cell growth (breast and melanoma) and compound **B-13** (X = $N(C_8H_{17})_2$, Y = Ac, R' = Ac) for melanoma cell growth. These results support the assumption that cytotoxicity is strongly related to the overall lipophilicity of the molecule.

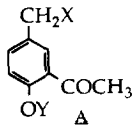
Even though the ligand–metal stability constants of oximes are ~10^4 times stronger in aqueous systems than those of the corresponding ketones, the activity of most of the oximes examined in this work towards inhibition of MCF-7 breast cancer cells and B-16 melanoma cells is only slightly higher than that of the corresponding ketones. In general, the oximes are more hydrophilic than the ketones, and those compounds where the X substituent is not hydrophobic enough (*eg*, X = OAc) will confer a lower cytotoxic activity for the oxime in comparison to the ketone. Of course compounds with long alkyl substituents, like **4** (X = OC_8H_{17}) and **9** (X = $N(C_8H_{17})_2$) are more lipophilic and possess a higher activity of the oximes in comparison to the ketones.

The acetates of **A-9** (**A-13**) and **B-9** (**B-13**) do show very strong cytotoxic effects which are identical to those of **A-9** and **B-9**. This surmises that these small lipophilic molecules can reach tumor sites, where they are hydrolyzed by esterase-type enzymes, and acquire their antitumor potency. Hence the activity of **A-13** is similar to the activity of **A-9** and the activity of **B-13** is similar to that of **B-9**. On the other hand, blocking



Scheme 1. Synthesis of ketones **A** and oximes **B**. For definition of R and X see table I.

Table I. Structure of ketones **A** and oximes **B** and inhibition of B16 melanoma cells.



Compound	*Substituent*			*% Inhibition*			
	X	*Y*	*R'*	*A (50 μM)*	*B (50 μM)*	*B (25 μM)*	*B (10 μM)*
A-1; B-1	OH	H	H		0		
A-2; B-2	OAc	H	H	24	17		
B-3	OAc	Ac	Ac		0		
A-4; B-4	OC_8H_{17}	H	H	32	92	23	17
A-5; B-5	$[O(CH_2)_2]_2OC_4H_9$	H	H	26	41	49	14
A-6; B-6	$OCH_2C_6H_3(COCH_3)(OH)$	H	H		0		
A-7; B-7	$N(C_3H_7)_2$	H	H	47	8		
A-8; B-8	$N^+(C_3H_7)_2CH_3I^-$	H	H				
A-9; B-9	$N(C_8H_{17})_2$	H	H	62	93	88	44
B-10	$N^+(C_8H_{17})_2CH_3I^-$	H	H		73	45	42
B-11	OC_8H_{17}	M = Fe^{2+}	H		12		
		M = Fe^{3+}			33		
		M = Zn^{2+}			17		
		M = Cu^{2+}			14		
A-13; B-13	$N(C_8H_{17})_2$	Ac	Ac	66	90	67	50

Initial cell number was 4 x 10^4/plate. 100% refers to 1.57–1.96 x 10^5 cells, counted following 48 h incubation of untreated cells. Each compound was tested in 2–3 independent experiments performed in triplicate. Concentration A was 50 μM, concentration B was 10, 25 or 50 μM.

Table II. Effect (%) of 2-hydroxyacetophenones and corresponding oximes on MCF-7 breast carcinoma (% inhibition).

Compound[a]	*Substituent*			*% Inhibition*		
	X	*Y*	*R'*	*10 μM*	*25 μM*	*50 μM*
A-4	OC_8H_{17}	H	H	31	44	44
B-4	OC_8H_{17}	H	H	37	49	88
A-5	$[O(CH_2)_2]_2OC_4H_9$	H	H	0	35	30
B-5	$[O(CH_2)_2]_2OC_4H_9$	H	H	36	53	65
A-9	$N(C_8H_{17})_2$	H	H	39	54	59
B-9	$N(C_8H_{17})_2$	H	H	49	88	84

Initial cell number was 1 x 10^5/plate. 100% refers to 2.73 x 10^5 ± 0.18 x 10^5 cells counted following 72 h of incubation of untreated cells. [a]For compound designation see table I.

the metal-binding ability by forming the metal complexes of **B-4** (**B-11**) considerably reduces the cytotoxic effect. Assuming that the cytotoxicity is related to the metal-chelating ability towards transition metal ions, blocking this ability causes a decrease in the effect.

In conclusion, the results obtained indicate that the lipophilic properties of the compounds and metal-chelating strength are favorable for cytotoxicity. The mechanism of the cytotoxicity is far from being known.

An important factor in the development of anti-proliferative compounds and drugs is their toxicity to

normal cells. Most cytotoxic drugs are toxic to all other normal cells yet their cardiotoxicity is one of the most worrisome features. Cardiotoxicity was therefore investigated, and it was shown that all the active antitumor agents of the oxime class are also strongly cardiotoxic. This does not necessarily mean that nontoxic 2-hydroxyacetophenones (**A**) or oximes (**B**) cannot be designed or found. But at this stage, metal chelators of high antitumor activity and low cardiotoxicity have been identified (Warshawsky and Nordenburg, unpublished results), and we are proceeding with synthesis and investigations of this class of chelators.

Experimental protocols

Materials and methods

All organic solvents were AR grade or distilled solvents. TLCs were performed on Merck Kieselgel 60F 254 plates with the eluents: EtOAc/hexane and $CHCl_3$/MeOH/NH_3(aq). The dying reagents were basic aqueous 1% $KMnO_4$ solution and ethanolic 0.2% ninhydrin solution.

Flash chromatography was carried out on 0.040–0.063 nm silica gel 60 (Merck No 9835). Melting points were determined on a Fisher-Johns apparatus. IR spectra were obtained on a Nicolet 510 FTIR spectrometer. ^{1}H NMR spectra were recorded on a Varian FT80A or Bruker 270 MHz Aspect 2000 spectrometers. Mass spectra were recorded on GC-MS Finnigan 4500.

All compound except **A13–B13** were analyzed by ^{1}H NMR, IR, GC-MS and elemental analyses. The precursor compounds to the acetate compounds **A-2**, **B-2**, **A-9** and **B-9** gave satisfactory elemental analyses and the acetate products **A-13** and **B-13** themselves gave good NMR spectra. The metal complexes were prepared by extraction. The metal/ligand ratio in the Cu^{2+} complex (**B-11**) was determined from the atomic absorption analyses of the residual metal solutions, using Varian 1000 atomic absorption spectrophotometer.

Cell proliferation experiments

B16 murine melanoma and MCF-7 human breast cancer cells were grown in RPMI 1640, supplemented with fetal calf serum (10%) and antibiotics (Biological Industries) at 37°C, in a humidified atmosphere of 95% air and 5% CO_2. Cells were transferred every 2–3 d, following trypsinization.

B16 melanoma cells (5 x 10^4) were incubated in 1 mL growth medium for 48 h, and breast cancer cells (10^5) for 72 h in tissue culture dishes (3 cm), in the absence and presence of the examined compounds. Following incubation, cells were detached with EDTA (1 mM in phosphate-buffered saline) and counted in a Coulter counter as previously described by us [1, 5]. Cell growth in the absence of treatment following 48 and 72 h for B16 melanoma and MCF-7 breast cancer cells respectively, served as the positive control. This value was used as 100%.

Beating cardiomyocyte cultures were prepared from rat embryonic hearts as previously described [9]. The cultures were incubated in the absence and presence of 25 μM of the agents for 24 and 48 h. The beats were counted under an inverted phase microscope. Treatment of cell cultures resulted in a negative chronotropic effect or complete arrest of beats. (Untreated cultures gave 120 beats/min.)

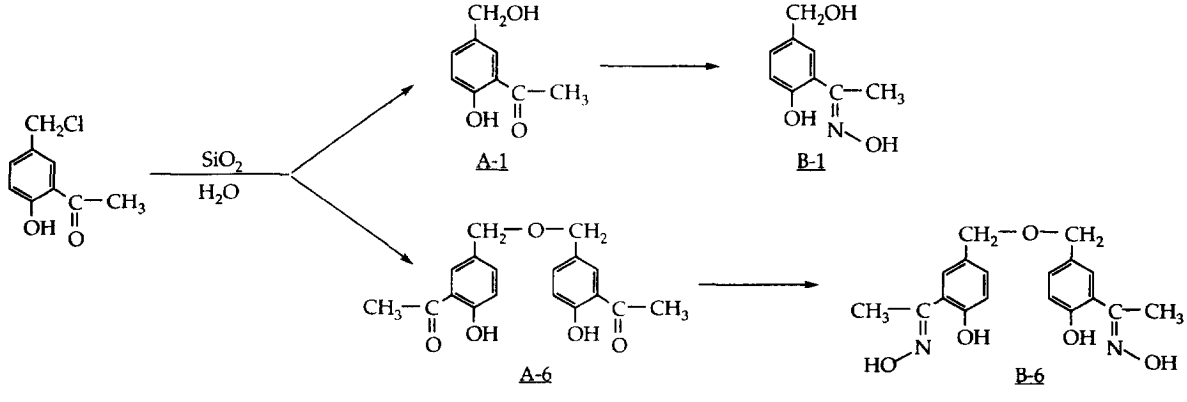
Chemistry

Etherification and amination of 2-acetyl-4-chloromethylphenol. A general procedure

2-Hydroxy-5-chloromethylacetophenone (1 mmol), the alcohol or amine (1 mmol) and sodium carbonate (0.75 mmol) were refluxed in $CHCl_3$ (1 mL) for 20 h (in the case of amine) or 48–70 h (in the case of alcohol). The reaction mixture was then filtered and evaporated and the crude product obtained was purified by flash chromatography on silica with EtOAc/hexane (10–30% of EtOAc) as the eluent.

Oximation. A general procedure

Hydroxylamine hydrochloride (1 mmol) and sodium bicarbonate (1 mmol) were dissolved in distilled water (0.15 mL) by warming to 30°C. The ketone (0.5 mmol) in absolute methanol (0.5 mL) was added and the reaction mixture was stirred at rt to 80°C for 24 h. $CHCl_3$ was then added, the mixture filtered, dried over Na_2SO_4 and evaporated. The crude product was puri-



Scheme 2. Synthesis of compounds **A-1**, **B-1**, **A-6** and **B-6** by silica catalysis.

fied by flash chromatography on silica with EtOAc/hexane (20–30% of EtOAc) as the eluent.

2-Hydroxy-5-(hydroxymethyl)acetophenone ***A-1***
This compound was prepared in the literature [10], our procedure is different. For preparation of wet silica, SiO_2 (70.3 g) was mixed with an H_2O (3.5 mL) and EtOAc (300 mL) mixture. The solvents were removed under vacuum by an evaporator.

2-Hydroxy-5-chloromethylacetophenone (0.200 g) in 50% EtOAc/hexane (22 mL) was added to the wet silica (10 g) and the solvents were evaporated. The loaded silica, after 4.5 h of standing was loaded on silica column and purified by flash chromatography with 50% EtOAc/hexane as the eluent to yield the product: 2-hydroxy-5-hydroxymethylacetophenone as an oil (0.041 g, 23%) which solidified with time. The product was crystallized from hexane, mp = 38–39°C; TLC: R_f = 0.32; 50% EtOAc/hexane.

^{1}H NMR: δ ($CDCl_3$) = 12.20 (1H, s, PhO*H*); 7.70 (1H, d, J = 2.1 Hz, H_3); 7.43 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.1 Hz, H_5); 6.90 (1H, d, J = 8.5 Hz, H_6); 4.60 (2H, s, CH_2OH); 2.60 (3H, s, $COCH_3$); 2.43 (1H, bs, CH_2OH). GC-MS: m/e = 166 (M^+, 48.8%), 152 (8.6%), 151 (100%), 123 (10.9%), 106 (6.7%), 105 (52.1%), 91 (14.6%), 77 (45%). $C_9H_{10}O_3$ (MW = 166.18) requires: C65.05, H6.07; found: C65.38, H6.08.

2-Hydroxy-5-(hydroxymethyl)acetophenoneoxime ***B-1***
Yield: 0.078 g; 78%; crystallization from EtOAc/hexane, mp = 161–163°C; TLC: R_f = 0.67, 80% EtOAc/hexane. MS: m/e = 164 (M-OH, 26%). ^{1}H NMR: δ (MeOD-d_4) = 7.45 (1H, d, J = 2.0 Hz, H_3); 7.20 (1H, dd, J_1 = 8.3 Hz, J_2 = 2.1 Hz, H_5); 6.83 (1H, d, J = 8.3 Hz, H_6); 4.53 (2H, s, CH_2OH); 2.31 (3H, s, CH_3). $C_9H_{11}NO_3$ (MW 181.91) requires: C59.66, H6.12, N7.73 found: C59.56, H6.16, N 7.82.

2-Hydroxy-5-(acetoxymethyl)acetophenone ***A-2***
Potassium acetate (1.45 g, 14.8 mmol) was added to the solution of 2-hydroxy-5-chloromethylacetophenone (1.00 g, 5.4 mmol) in 1,4-dioxane (12 mL). The reaction mixture was placed in a silicon oil bath and refluxed for 20 h. The solvent was evaporated, $CHCl_3$ and distilled H_2O were added and the two phases were separated after shaking. The organic phase was washed with H_2O, dried over Na_2SO_4, filtered and evaporated to obtain a brownish solid which was crystallized from hot hexane to yield the product as white crystals (0.90 g, 80%), mp = 61–64°C; TLC: R_f = 33% EtOAc/hexane.

^{1}H NMR: δ ($CDCl_3$) = 12.27 (1H, s, PhO*H*; 7.73 (1H, d, J = 2.1 Hz, H_3); 7.49 (1H, dd, J_1 = 8.6 Hz, J_2 = 2.1 Hz, H_5); 6.97 (1H, d, J = 8.5 Hz, H_6); 5.04 (2H, s, CH_2OAc); 2.64 (3H, s, $COCH_3$); 2.08 (3H, s, O_2CCH_3). $C_{11}H_{12}O_4$ (MW = 208.21) requires: C63.45, H5.81; found: C63.35, H5.92.

2-Hydroxy-5-(acetoxymethyl)acetophenoneoxime ***B-2***
Hydroxylamine hydrochloride (0.278 g, 4 mmol) and sodium bicarbonate (0.336 g, 4 mmol) were dissolved in distilled H_2O (0.5 mL) by warming to 40°C. The ketone (0.416 g, 2 mmol) in methanol (1.5 mL) was added and the reaction mixture was stirred for 20 h at room temperature, then chloroform added to the milky solution, the phases separated and the organic phase dried over Na_2SO_4, filtered and evaporated. The oil obtained (0.345 g) was purified by flash chromatography on silica with 30% EtOAc/hexane as the eluent to obtain the product (0.130 g, 30%) as a white solid. Crystallization from CH_2Cl_2/hexane, mp = 108–109°C; TLC: R_f = 0.46; 33% EtOAc/hexane.

^{1}H NMR: δ (CD_2Cl_2) = 11.26 (1H, s, PhO*H*); 7.77 (1H, s, NO*H*); 7.45 (1H, d, J = 2.1 Hz, H_3); 7.26 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, H_5); 6.90 (1H, d, J = 8.4 Hz, H_6); 5.02 (2H, s, $PhCH_2O_2C$); 2.35 (3H, s, $CNCH_3$); 2.05 (3H, s, $COCH_3$). $C_{11}H_{13}NO_4$ (MW = 223.23) requires: C59.18, H5.87, N6.28; found: C59.02, H5.91, N5.98.

2-Acetoxy-5-(acetoxymethyl)acetophenoneoxime acetate ***B-3***
The oxime **B-2** (50 mg) was stirred for 2.5 h with acetic anhydride (1 mL) at 150°C followed by stirring for an additional 20 h at rt. The acetic anhydride was then removed under high vacuum (60°C) to obtain a brownish oil (67 mg, 97%) which was crystallized from EtOAc/hexane to yield the product as an off-white powder, mp = 102–104°C; TLC: R_f = 0.25, 33% EtOAc/hexane.

^{1}H NMR: δ (CD_2Cl_2) = 7.50–7.35 (2H, m, H_3 + H_5); 7.13 (1H, d, J = 8.9 Hz, H_6); 5.09 (2H, s, $PhCH_2O_2C$); 2.29, 2.26, 2.19, 2.07 (12H, 4s, 4 x CH_3). $C_{15}H_{17}NO_6$ (MW = 307.30) requires: C58.62, H5.58, N4.56; found: C58.83, H5.58, N4.86.

2-Hydroxy-5-(octyloxymethyl)acetophenone ***A-4***
Oil (1.35 g, 32%) TLC: R_f = 0.60, 20% EtOAc/hexane. ^{1}H NMR: δ ($CDCl_3$) = 12.22 (1H, s, PhO*H*); 7.70 (1H, d, J = 1.9 Hz, H_3); 7.44 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, H_5); 7.95 (1H, d, J = 8.5 Hz, H_6); 4.43 (2H, s, $PhCH_2O$); 3.47 (2H, t, J = 6.2 Hz, $PhCH_2OCH_2$); 2.63 (3H, s, $COCH_3$); 1.7–1.4 (2H, m, $OCH_2CH_2CH_2$); 1.27 (10H, s, $(CH_2)_5$); 0.87 (3H, bt, CH_3). See reference [3], compound **23**.

2-Hydroxy-5-(octyloxymethyl)acetophenoneoxime ***B-4***
Yield: 0.84 g, 83%, mp = 56–56.5°C; TLC: R_f = 0.42, 20% EtOAc/hexane. ^{1}H NMR: δ ($CDCl_3$) = 7.41 (1H, d, J = 2.0 Hz, H_3); 7.23 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, H_5); 6.94 (1H, d, J = 8.3 Hz, H_6); 4.45 (2H, s, $PhCH_2O$); 3.47 (2H, t, J = 6.7 Hz, OCH_2CH_2); 2.34 (3H, s, $CNCH_3$); 1.6 (2H, m, OCH_2CH_2); 1.26 (10H, bs, $(CH_2)_5$); 0.87 (3H, t, CH_3) (recorded on a Bruker 270 MHz Aspect 2000 spectrometer). See reference [3], compound **23a**.

2-Hydroxy-5-[2-(2-butoxyethoxy)ethoxymethyl]acetophenone ***A-5***
Oil (0.064 g, 10%); TLC: R_f = 0.30, 33% EtOAc/Hexane. ^{1}H NMR: δ ($CDCl_3$) = 12.22 (1H, s, PhO*H*); 7.72 (1H, d, J = 2.0 Hz, H_3); 7.45 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.1Hz, H_5); 6.84 (1H, d, J = 8.5 Hz, H_6); 4.51 (2H, s, $PhCH_2O$); 3.67, 3.62 (8H, 2s, $[OCH_2CH_2]_2O$); 3.45 (2H, t, J = 6.4 Hz, $OCH_2(CH_2)_2CH_3$); 2.63 (3H, s, $COCH_3$); 1.8–1.1 (4H, m, $CH_2)_2$ CH_3); 0.90 (3H, t, CH_3). GC-MS: m/e = 310 (M^+). IR: ν_{max} = 1104 cm^{-1} (C-O-C). See reference [3], compound **26**.

2-Hydroxy-5-[2-(2-butoxyethoxy)ethoxymethyl] acetophenoneoxime ***B-5***
Yield: (149 mg, 88%); TLC: R_f = 0.31, 33% EtOAc/hexane. ^{1}H NMR: δ (CD_2Cl_2) = 8.38 (1H, s, PhO*H*); 7.40 (1H, d, J = 1.9 Hz, H_3); 7.22 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, H_5); 6.86 (1H, d, J = 8.3 Hz, H_6); 4.47 (2H, s, $PhCH_2O$); 3.63; 3.58 (8H, 2s, $(OCH_2CH_2]_2O$); 3.43 (2H, t, J = 6.3 Hz, $OCH_2C_3H_7$); 2.30 (3H, s, $CNCH_3$); 1.6–1.1 (4H, m, $(CH_2)_2CH_3$); 0.89 (3H, t, CH_3). GC-MS: m/e = 308 (M^+-OH). IR: ν_{max} = 1095 cm^{-1} (C-O-C). See reference [3], compound **26a**.

5,5'-Oxidodimethyl-bis-(2-hydroxyacetophenone) ***A-6***
The synthesis is similar to that of 2-hydroxy-5-hydroxymethylacetophenone **A-1**. The hydroxymethyl and the ether are both the products of the silica-catalyzed reaction of the 2-hydroxy-5-chloromethylacetophenone. 2-Hydroxy-5-chloromethylacetophenone (0.100 g) in 50% EtOAc/hexane (20 mL) was loaded

on wet silica (10 g) . The mixture was left standing for 16 h, then loaded on a silica column and separated by flash chromatography with 50% EtOAc/hexane as the eluent to obtain the product as white solid (0.020 g, 24%), mp = 96–98°C, TLC: R_f = 0.65, 50% EtOAc/hexane.

^{1}H NMR: δ ($CDCl_3$) = 12.25 (2H, s, PhO*H* x 2); 7.70 (2H, d, J = 2.1 Hz, H_3 x 2); 7.46 (2H, dd, J_1 = 8.6 Hz, J_2 = 2.1 Hz, H_5 x 2); 6.96 (2H, d, J = 8.6 Hz, H_6 x 2); 4.50 (4H, s, PhCH_2O); 2.63 (6H, s, COCH_3 x 2). GC/MS: *m/e* = 314 (M^+, 0.6%), 178 (7.8%), 150 (43.3%), 149 (100%), 142 (8.8%), 135 (33.75%), 107 (55.23%), 91 (6.67%), 78 (15.7%), 77 (72.24%), 65 (20.77). $C_{18}H_{15}O_5$ (MW 314.341) requires: C68.78, H5.77; found: C68.46, H5.85.

5,5'-Oxidodimethyl-bis (2-hydroxyacetophenoneoxime) ***B-6***
Hydroxylamine hydrochloride (62 mg, 0.9 mmol) and sodium bicarbonate (75 mg, 0.9 mmol) were dissolved in distilled H_2O (0.4 mL). The diketone (47 mg, 0.15 mmol) in methanol (0.4 mL) was added and the reaction mixture was stirred for 17 h at 76°C, then $CHCl_3$ was added to the milky solution obtained, the two phases separated and the organic phase dried over Na_2SO_4 and evaporated. The white powder obtained (46 mg) was purified by flash chromatography on silica with 33% EtOAc/hexane as the eluent to yield the product (30 mg, 58%) as white powder, mp = 194–197°C; TLC: R_f = 0.60, 50% EtOAc/hexane.

^{1}H NMR: δ (MeOD-d_4) = 7.44 (2H, d, J = 2.0 Hz, H_3); 7.21 (2H, dd, J_1 = 8.4 Hz, J_2 = 1.9 Hz, H_5); 6.84 (2H, d, J = 8.3 Hz, H_6); 4.47 (4H, s, PhCH_2O x 2); 2.30 (6H, s, CNCH_3 x 2). $C_{18}H_{20}N_2O_5$ (MW = 344.36) requires: C62.78, H5.85, N8.14; found: C61.52, H5.91, N7.94.

2-Hydroxy-5-(dipropylaminomethyl)acetophenone ***A-7***
Yield: (3.9 g, 94%); TLC: R_f = 0.4, 30% EtOAc/hexane. ^{1}H NMR: δ ($CDCl_3$) = 12.15 (1H, s, PhO*H*); 7.70 (1H, d, J = 1.9 Hz, H_3); 7.43 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.2 Hz, H_5); 6.90 (1H, d, J = 8.5 Hz, H_6); 3.48 (2H, s, PhCH_2N); 2.62 (3H, s, COCH_3); 2.38 (4H, t, J = 7.2 Hz, NCH_2CH$_2$ x 2); 1.7–1.1 (4H, m, NCH_2CH$_2$ x 2); 0.86 (6H, t, J = 6.9 Hz, CH_3 x 2). $C_{15}H_{23}NO_2$ (MW = 249.34) requires: C72.25, H9.30, N5.62; found: C70.85, H9.46, N5.44.

2-Hydroxy-5-(dipropylaminomethyl)acetophenoneoxime ***B-7***
The oily solid (1.79 g, 72%) was crystallized from hexane to obtain white crystals, mp = 105–106°C. TLC: R_f = 0.32, 30% EtOAc/hexane; ^{1}H NMR: δ (CD_2Cl_2) = 7.46 (1H, d, J = 2.0 Hz, H_3); 7.14 (1H, dd, J_1 = 8.3 Hz, J_2 = 2.0 Hz, H_5); 7.80 (1H, d, J = 8.2 Hz, H_6); 3.56 (2H, s, PhCH_2N); 2.55–2.20 (4H, m, NCH_2CH$_2$ x 2); 2.32 (3H, s, CNCH_3); 1.7–1.3 (4H, m, NCH$_2$CH_2); 0.87 (6H, t, J = 7.0 Hz, CH_3 x 2). $C_{15}H_{24}N_2O_2$ (MW = 264.35) requires: C68.15, H9.15, N10.60; found: C68.45, H9.34, N10.53.

2-Hydroxy-5-(dipropyl(methyl)ammoniomethyl)acetophenone-oxime iodide ***B-8***
The oxime **B-7** (0.302 g, 1.14 mmol) was dissolved in absolute EtOH (1.2 mL) by heating. After cooling, CH_3I (0.15 mL, 2.3 mmol) was added and the reaction mixture was stirred for 18 h at room temperature. The heavy white precipitate obtained was filtered under vacuum, washed with cold absolute EtOH (dried over CaO) and dried under high vacuum to obtain the product as a white powder (0.340 g, 73%), which was crystallized from EtOH/CH_2Cl_2, mp = 170–172°C; TLC: R_f = 0, 50% EtOAc/hexane.

^{1}H NMR: δ (MeOD-d_4) = 7.68 (1H, d, J = 2.2 Hz, H_3); 7.42 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.3 Hz, H_5); 7.03 (1H, d, J = 8.4 Hz, H_6); 4.54 (2H, s, PhCH_2N⊕); 2.99 (3H, s, N⊕CH_3); 2.39 (3H, s, CNCH_3); 2.1–1.6 (4H, m, NCH$_2$CH_2 x 2); 1.05 (6H, t, J = 7.1 Hz, CH_3 x 2). $C_{16}H_{27}N_2O_2I$ (MW = 406.31) requires: C47.29, H6.70, N6.90; found: C47.26, H6.76, N 6.85.

2-Hydroxy-5-(dioctylaminomethyl)acetophenone ***A-9***
To 2-hydroxy-5-chloromethylacetophenone (2.767 g, 15 mmol) in $CHCl_3$ (10 mL) was added Na_2CO_3 (0.9 g, 8 mmol) followed by dioctylamine (3.622 g, 15 mmol) in $CHCl_3$ (10 mL). The reaction mixture was refluxed for 25 h and the solid was then filtered off. The filtrate was washed with 5% $NaHCO_3$, dried over Na_2SO_4 and evaporated to obtain 6.4 g of yellowish oil. The oil was purified by flash chromatography on silica with 10% EtOAc/hexane as the eluent to yield the product as yellowish oil (4.14 g, 71%). TLC: R_f = 0.50, 20% EtOAc/hexane.

^{1}H NMR: δ ($CDCl_3$) = 10.98 (1H, s, PhO*H*); 7.69 (1H, s, H_3); 7.43 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.1 Hz, H_5); 6.92 (1H, d, J = 8.5 Hz, H_6); 3.48 (2H, s, PhCH_2N); 2.64 (3H, s, COCH_3); 2.38 (4H, t, J = 7.1 Hz, NCH_2CH$_2$ x 2); 1.44 (4H, m, NCH$_2$CH_2 x 2); 1.25 (20H, s, (CH_2)$_5$ x 2); 0.87 (6H, t, J = 6.3 Hz, CH_3 x 2). See reference [3] compound **20**.

2-Hydroxy-5-(dioctylaminomethyl)acetophenoneoxime ***B-9***
Hydroxylamine hydrochloride (1.806 g, 26 mmol) and sodium bicarbonate (2.182 g, 26 mmol) were dissolved in distilled water (2.5 mL) by warming to 40°C. The ketone (2.53 g, 6.5 mmol) in absolute MeOH (5 mL) was added and the mixture was stirred at 70°C for 24 h. Chloroform was then added, the two phases separated, the oranic phase washed with 1 N H_2SO_4 followed by saturated NaCl and by 5% $NaHCO_3$, dried over Na_2SO_4 and evaporated. The oily solid obtained was purified by flash chromatography on silica with 20% EtOAc/hexane as the eluent, to obtain the product (2.41 g, 92%) as an oily solid. TLC: R_f 0.40, 20% EtOAc/hexane.

^{1}H NMR: δ ($CDCl_3$) = 11.36 (1H, bs, PhO*H*); 7.42 (1H, d, J = 1.7 Hz, *Ha*); 7.12 (1H, dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, *Hc*); 7.84 (1H, d, J = 8.2 Hz, *Hb*); 3.54 (2H, s, PhCH_2N); 2.6–2.2 (4H, m, NCH_2CH$_2$ x 2); 2.31 (3H, s, CNCH_3); 2.7–2.3 (4H, m, NCH$_2$CH_2 x 2); 1.25 (20H, s, (CH_2)$_5$ x 2); 0.85 (6H, t, J = 4.9 Hz, CH_3 x 2). See reference [3], compound **20a**.

2-Hydroxy-5-(dioctyl(methyl)ammoniomethyl)acetophenone-oxime iodide ***B-10***
Methyl iodide (0.07 mL, 1 mmol) was added to the oxime **B-9** (0.207 g, 0.5 mmol) solution in absolute EtOH (1.2 mL, dried over CaO). The reaction mixture was stirred 22 h at rt followed by additional 2.5 h at 50°C, and then cooled overnight in the refrigerator. The white precipitate obtained was filtered and washed with cold absolute EtOH to obtain the product (0.098 g). The mother liquor was evaporated to 0.5 mL, cooled in refrigerator to receive an additional 0.058 g of the product. Total yield 0.156 g, 56%. The product was crystallized from CH_2Cl_2/hexane, mp = 153–154°C.

^{1}H NMR: 7.62 (1H, bs, H_3); H_5 hidden under $CHCl_3$ peak; 6.69 (1H, d, J = 8.4 Hz, H_6); 4.80 (2H, bs, PhCH_2N⊕); 3.5–3.0 (4H, m, N⊕CH_2CH$_2$); 3.10 (3H, s, N⊕CH_3); 2.22 (3H, s, CNCH_3); 2.0–1.5 (4H, m, NCH$_2$CH_2); 1.29 (20H, s, (CH_2)$_5$ x 2); 0.87 (6H, bt, CH_3 x 2). $C_{26}H_{47}N_2O_2I$ (MW = 546.57) requires: C57.13, H8.67, N 5.13; found: C57.38, H8.80, N5.17.

Metal complexes of 2-hydroxy-5-(octyloxymethyl)acetophenone-oxime ***B-11***
The oxime solution 0.002 M in $CHCl_3$ (70 mL) was stirred for 10 min with 0.02 M MSO_4 (M = Cu, Fe, Zn) or $FeCl_3$ in 0.2 M acetate buffer pH = 4.0 (70 mL). Then the aqueous phase was

removed and a fresh M^{n+} solution was added (0.02 M, 70 mL) and the mixture stirred for another 10 min. This step was repeated once more. The two phases were separated and the combined organic phases dried over Na_2SO_4, filtered and evaporated. The complexes oxime•M^{n+} were obtained for M^{n+} = Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+} as powders.

The ratio oxime/metal in the Cu complex was found to be two. It was established by extraction of the Cu complex (4 mg) in $CHCl_3$ (5 mL) by 2 N H_2SO_4 (5 mL) twice. The combined aqueous reextracts were diluted to 250 mL and the Cu^{2+} content was measured by an atomic absorption spectrometer (1.4 ppm, 5.5 μmol Cu^{2+}) (ratio oxime/metal = [4 x 10^{-3}/5.5 x 10^{-6} – (63.5 – 2)]/293.2 = 2.27).

2-Acetoxy-5-(dioctylaminomethyl)acetophenone ***A-13***

The ketone **A9** (0.50 g, 1.28 mmol) was stirred with acetic anhydride (4 mL) at 150°C for 21 h, and then the acetic anhydride was removed by high vacuum and the mixture was purified by flash chromatography on silica with 10% EtOAc/hexane as the eluent to yield the product (0.140 g, 25%). TLC: R_f = 0.56, 20% EtOAc/hexane.

^{1}H NMR: δ ($CDCl_3$) = 7.78 (1H, d, J = 1.7 Hz, H_3); 7.5 (1H, dd, H_5); 7.13 (1H, d, H_6); 3.55 (2H, s, PCH_2N); 2.54 (3H, s, $PhCOCH_3$); 2.32 (3H, s, PhO_2CH_3); 2.6–2.2 (4H, m under PhO_2CCH_3 peak, CH_2CH_2 x 2); 1.25 (24H, s, $(CH_2)_6$ x 2); 0.87 (6H, bt, CH_3 x 2).

2-Acetoxy-5-(dioctylaminomethyl)acetophenoneoxime acetate ***B-13***

The oxime (0.100 g, 0.25 mmol) in acetic anhydride (2 mL) and pyridine (1 mL) was stirred for 18 h at rt, then distilled H_2O added and the mixture extracted twice with $CHCl_3$. The organic phase washed with 0.5 N HCl followed by NaCl (sat), dried over Na_2SO_4, filtered and evaporated to receive the product as an oil (0.230 g).

^{1}H NMR: δ ($CDCl_3$) = 7.95 (1H, d, J = 1.7 Hz, H_3); 764 (1H, dd, J_1 = 8.4 Hz, J_2 = 1.9 Hz, H_5); 7.16 (1H, d, J = 8.2 Hz, H_6); 4.06 (2H, s, $PhCH_2N^{\oplus}H$); 3.0–2.65 (4H, m, $HN^{\oplus}CH_2CH_2$ x 2); 2.38, 2.30, 2.21 (9H, 3s, CH_3 x 3); 1.95–1.50 (4H, m, NCH_2CH_2 x 2); 1.26 (20H, s, $(CH_2)_5$ x 2); 0.87 (6H, t, CH_3 x 2).

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