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TETRAHEDRON: *ASYMMETRY*

Synthesis and functionalization of chiral superstructured porphyrins

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Abstract

Chiral porphyrins **1** and **2**, bearing, respectively, four and two chains at the *meso* carbons, each one with a stereocentre at C-2 and a double bond at C-5, were functionalized to afford the corresponding thiosemicarbazonic derivatives **9**–**12** and the nickel(II) porphyrinato **13**, compounds with potential antiviral, antibacterial and antitumour properties. To verify the biological activity of some of these products, assays of proliferation inhibition and apoptosis tests in vitro on human leukemic cell lines U937 were carried out for compounds **1**, **6**, **9** and **10**.

1. Introduction

Natural porphyrins (such as cytochrome P_{450} enzymes, chlorophyll, haemoglobin, etc.)[1] exhibit different functions since they present diverse porphyrinic contours. Therefore changing the structure of the synthetic porphyrins to try to produce compounds able to mimic the behaviour of the widely spread natural porphyrinic macrocycles and to interact with biomolecules or cells seems to be of great importance.

In recent years we have synthesized water-soluble and amphiphilic C-glyco-porphyrins.[2] In this paper the construction and the functionalization of the superstructured macrocycles **1** and **2** (Schemes 1 and 2) bearing, respectively, four and two chains at the *meso* carbons, each one with a stereogenic centre at C-2 and a double bond at C-5, is reported. We believe that the introduction of chirality and of a double bond in the *meso* substituents is important because of its possible transformation either into two hydroxyls,

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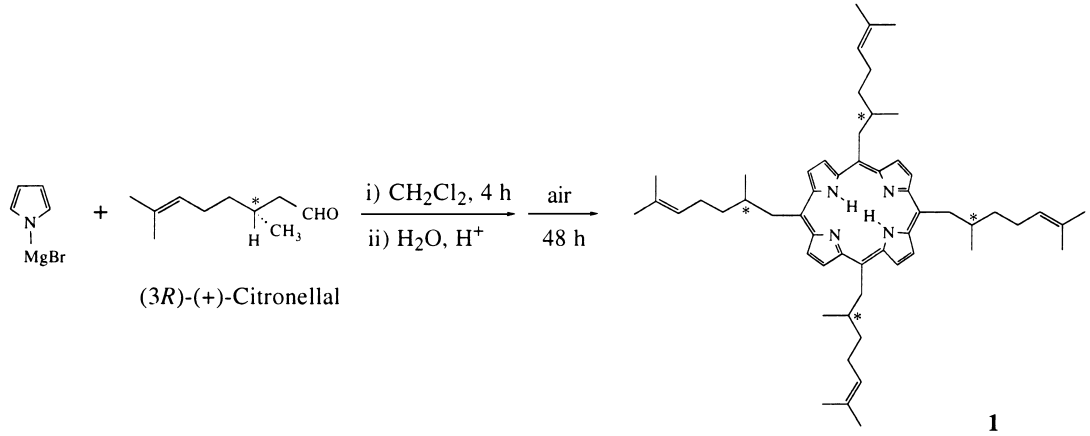
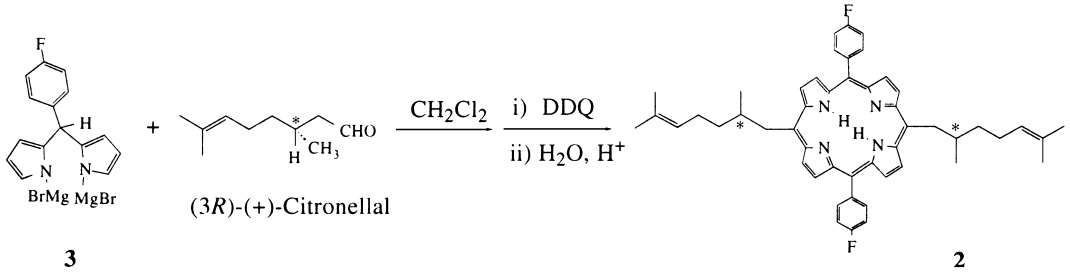
that could confer amphiphilic properties on the whole molecule, or into an aldehydic group, that could be functionalized to give thiosemicarbazonic compounds, of which the antitumour and antiviral activities are known.[3]



Scheme 1.



Scheme 2.

2. Results and discussion

2.1. Synthesis of the porphyrins *1* and *2*

At first we studied the length of the *meso* chain to bring the functional groups near a metal into the porphyrinic cavity. Among chiral aldehydes of appropriate length we chose (3*R*)-(+)-citronellal, a natural (C_{10}) aldehyde, which presents a stereogenic centre at C-3 and a double bond at C-6.

The reaction between pyrrole and a natural aldehyde such as (3*R*)-(+)-citronellal strangely occurred in low yield with $BF_3 \cdot OEt_2$ under Lindsey's conditions.[4] However, by using pyrrolylmagnesiumbromide and (3*R*)-(+)-citronellal in CH_2Cl_2 under argon and subsequent air oxidation, porphyrin **1**, bearing at the *meso* carbons four tentacles with a stereogenic carbon at C-2 and a double bond at C-5, was obtained in one step and in acceptable yield (7.8%, Scheme 1). The reaction mechanism is analogous to those proposed in our previous studies on the selective arylation and hetero-arylation of protected carbohydrates.[2]

Due to its acidity and strong coordinating capability, $MgBr^+$ is an ideal promoter to enhance the electrophilic power of the aldehydic carbon, the nucleophilicity of the pyrrole C-2 carbon, and, last but not least, minimize N-attack by ensuring C-2 regioselectivity. Moreover, $MgBr^+$ is able to coordinate

four or five electron-rich atoms, so that the complex geometry can favour, as in this particular case, the direct formation of the porphyrinogen. In fact pyrrolylmagnesiumbromide (10 mmol) and (3*R*)-(+)-citronellal (5 mmol, 1:2 molar ratio with respect to pyrrolylmagnesiumbromide) in 100 ml of CH_2Cl_2, under argon, afforded, after 4 h and by quenching the magnesiumbromide salt with a saturated NH_4Cl solution (H_2O/H^+), the porphyrinogen (not isolated). After a 48 h air oxidation and purification by flash chromatography separation on silica gel, compound **1** was obtained in 7.8% yield (Scheme 1).

Next we proposed a new superstructured porphyrin **2**, derived from citronellal, but bearing only two stereogenic chains in *meso* positions, to compare its properties with compound **1**. We needed to apply a different strategy to obtain porphyrin **2**.[5] The known compound 4′-fluoro-phenyldipyrrylmethane[5] was prepared and converted to 4′-fluoro-phenyldipyrrylmethanebismagnesiumbromide **3** using EtMgBr (2:1 molar ratio compared to the dimeric compound) in dry Et_2O (30 ml); the solvent was subsequently removed under vacuum, and anhydrous CH_2Cl_2 (60 ml) was added. Compound **3** (0.4 mmol) gave in the presence of (3*R*)-(+)-citronellal (0.4 mmol, 1:1 molar ratio), in 90 ml of CH_2Cl_2, under an argon stream, the porphyrinogen (not isolated), that, by DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) oxidation and following NH_4Cl (H_2O/H^+) quenching, afforded, after flash chromatographic purification, the porphyrinic macrocycle **2** in 4.7% yield (Scheme 2).

In conclusion, $MgBr^+$ appears to be an efficient promoter to the synthesis of new chiral porphyrins bearing at the *meso* carbons two or four chains able to be modified to obtain tetrapyrrolic macrocycles with different and useful functions.

2.2. Functionalization of the macrocycles

The introduction of a double bond in the *meso* substituents of porphyrins **1** and **2** could be particularly interesting: in fact the double bond can be transformed either into two hydroxyls, that could confer amphiphilic properties on the whole molecule, or into an aldehydic group, that could be functionalized to give thiosemicarbazonic compounds.[3] The synthetic path we followed consisted of an oxidation carried out on the double bond, with subsequent oxidative cleavage.[6]

2.3. Hydroxylation reaction

Porphyrin **1**, dissolved in anhydrous CH_2Cl_2, was subjected to hydroxylation with $KMnO_4$ and *cis*-dicyclo-hexane-18-crown-6 ether, under stirring at 263 K for 5 h, under a stream of argon.[6] The reaction, quenched by addition of a saturated Na_2SO_3 solution and of a 5% citric acid solution, was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and evaporated under vacuum, to afford, after purification by flash chromatography, the porphyrins **4** (12%), **5** (8%), **6** (22%), **7** (23%) and **8** (30%) as blue-fluorescent red-brown powders (Fig. 1). All products (**4**–**8**) were characterized by NMR, UV–vis techniques, mass spectrometry and elemental analysis.

The main feature of these compounds lies in the different number of hydroxylic functions present on the *meso*-substituents (two, four, six and eight), that confer to the macrocycles amphiphilic properties (**5** and **6**) and/or water-solubility (**7** and **8**). The introduction of the hydroxylic functions creates new stereocentres on the the side chains of the C-5 carbons, but the chirality of compounds **4**–**8** has not been determined since it is lost in the subsequent oxidative cleavage of the alcoholic group CHOH to aldehydic function CHO. The ^1H NMR spectra were very complicated, but the characterization of every single product isolated was possible by means of mass spectrometry, elemental analysis, and, above all, UV–vis techniques.

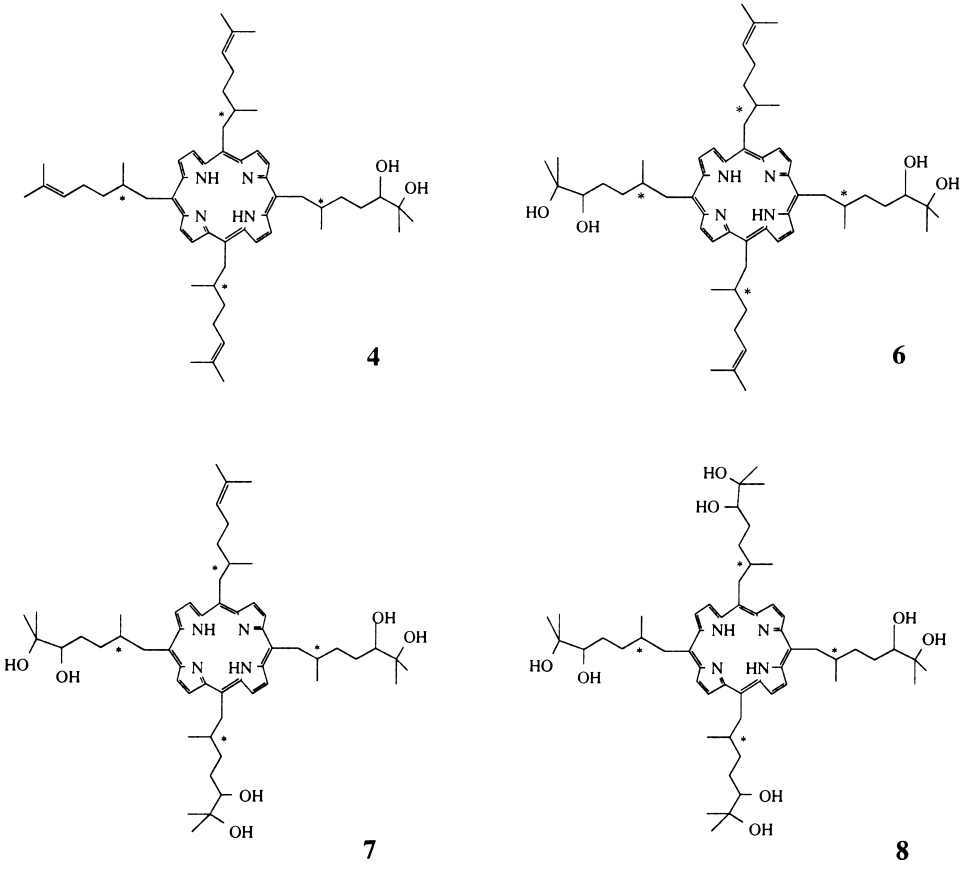


Figure 1. Principal hydroxylated porphyrins **4**–**8**

It is remarkable that porphyrin **8**, in which all the alkenylic functions were oxidized (Fig. 1), was the product obtained in the highest yield from this reaction (30%). Moreover, compound **8** was the only one produced when $KMnO_4$ was added in a 4:1 molar ratio with respect to the starting porphyrin **1**.

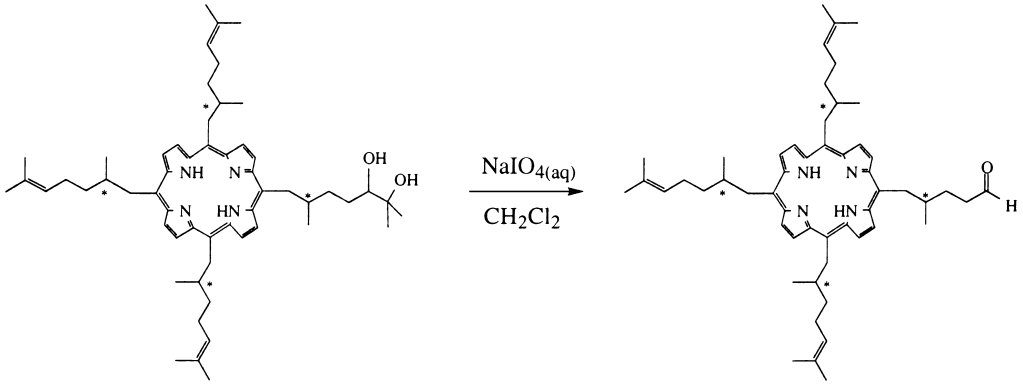
2.4. Oxidative cleavage reaction and subsequent condensation with thiosemicarbazide

The oxidative cleavage reaction with $NaIO_4$ was carried out on the four hydroxylated compounds obtained in reasonable yield: **4** (12%), **6** (22%), **7** (23%) and **8** (30%), to afford the corresponding aldehydes, that were immediately condensed with thiosemicarbazide to produce the porphyrinic macrocycles **9**–**12**, which bear a different number of thiosemicarbazonic residues in the *meso* positions.

The synthetic procedure is similar for all the oxidized porphyrins (**4**–**8**). For compound **4**, to an aqueous solution of $NaIO_4$, in a flask containing SiO_2 and CH_2Cl_2, was added, with stirring and at room temperature, the red solution of porphyrin **4** previously dissolved in CH_2Cl_2. After 1 h the reaction mixture was filtered, dried over Na_2SO_4, filtered and evaporated to give the corresponding porphyrin **14**, which has the aldehydic function in a *meso* position, as a green powder (55% yield, Scheme 3).

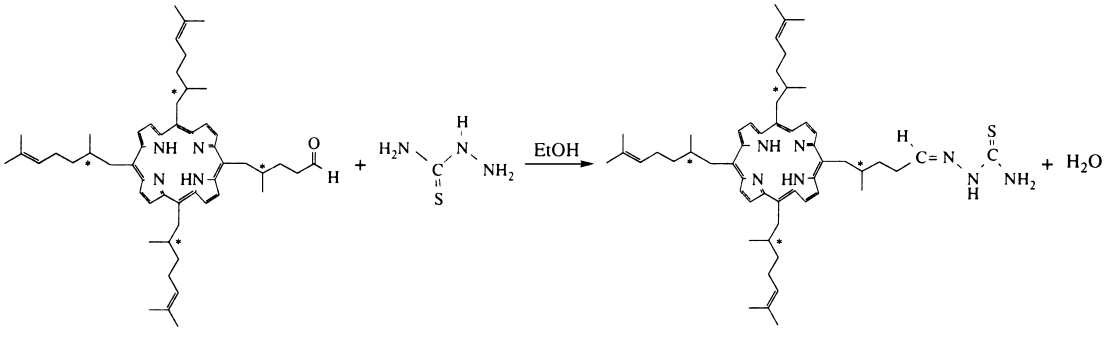
Compound **14** was immediately condensed with thiosemicarbazide (1:1 molar ratio with respect to porphyrin **14**) and dissolved in EtOH 95%, to give the thiosemicarbazonic derivate **9** as a bright yellow powder (98% yield, Scheme 4).

We proceeded in the same way for the other oxidized porphyrins **6**–**8**, suitably changing the



4 **14**

Scheme 3.



14 **9**

Scheme 4.

$NaIO_4$:macrocycle molar ratio (2:1, 3:1 and 4:1, respectively, for products **6**–**8**, in accordance with the number of hydroxylated side chains), and the amount of thiosemicarbazide. In this way we isolated porphyrins **10** (94% yield), **11** (91%) and **12** (91%), that present, in the same organic molecule, chemical species that are biologically active and possess different reaction mechanisms: in fact it is known that both porphyrins and thiosemicarbazones are able to carry out antiviral, antibacterial and antitumour activities.[3] So, as part of a research programme aimed at the synthesis and characterization of compounds with these properties, compounds **1**, **6**, **9** and **10** (Fig. 2) have been tested in vitro on human leukemic cell lines U937, focusing our attention on their activity with respect to cell proliferation inhibition and apoptosis induction.

2.5. Metallated-porphyrins

Metallated-porphyrins, bearing stereogenic substituents, can be used as catalysts in many reactions, such as asymmetric hydroxylations of hydrocarbons,[7] asymmetric epoxydations of unfunctionalized alkenes[8] and asymmetric sulphoxydations of sulphides.[9] Our main interest concerning metallated-porphyrins (porphyrinatoes) is mainly directed towards their possible application in the bio-inorganic field. The introduction of the metal into the porphyrinic cavity might create a model for a corresponding enzymatic system. So, after synthesizing and characterizing the macrocycles **1** and **2**, complexation

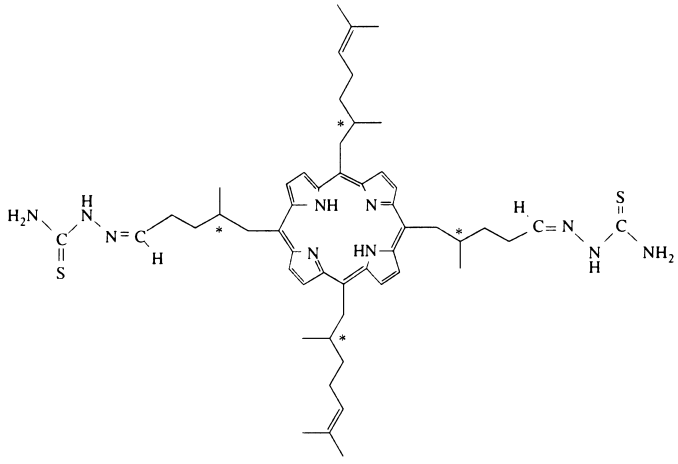


Figure 2. Porphyrin **10**

assays were carried out with inorganic salts of metals such as Cu, Co and Ni (cofactors in many enzymes) in different solvents, but powders (not crystals) were obtained. On trying to induce a regular stacking in the examined porphyrins, which is always difficult because of the porphyrin planarity deviation (see also the ^{1}H NMR spectroscopy paragraph),[10,11] we thought to functionalize them in the *meso* positions, so that the presence of new nucleophilic centres could establish coupled hydrogen bonds, able to stabilize the whole molecular system. We attempted this by condensation with the thiosemicarbazide, introducing on the porphyrinic side chains the new donor atoms N and S, but no crystals were obtained. We wanted to verify whether the metal ion could produce a major structural rigidity and then, in this way, favour crystallization. From a crystal structure the conformation of the molecule chelating the metal, the presence of coordinative insaturation sites, and the possible formation of hydrogen bonds coupled like the ones shown by the heterocyclic bases in the polynucleotidic DNA chain, would have been elucidated.

For this purpose we studied the reaction between the synthesized compound **9** and $Ni(Ac)_2 \cdot 4H_2O$. Porphyrin **9** carries nitrogen atoms both in the lipophilic cavity and in the *meso* substituent (thiosemicarbazide residue) near the sulfur atom, so the metal could be coordinated inside and/or outside the macrocycle cavity. From analysis of the ^{1}H NMR and the UV–vis spectra, using a 1:1 molar ratio of the correspondent metal acetate in a $CHCl_3$:MeOH 1:1 mixture,[12] the metal preferred to enter into the porphyrinic core, despite the donor atoms on the *meso* chain (Fig. 3). The formation of the metal complex (**13**, Fig. 3) produced a minor planarity deviation, as further confirmed by the UV–vis blue shifts of the Soret bands (414 nm versus 419 nm in the free macrocycle), and a greater stability, as known for many other complexes.[13]

2.6. UV–visible spectroscopy

A major feature of porphyrins, when irradiated between 200 and 700 nm, is the very strong absorbance at about 400 nm (Soret band) and less strong absorbances in the range between 500 and 650 nm (Q bands), due to electronic π–π transitions. UV–vis spectroscopy is thus very diagnostic and helpful for porphyrins. Absorbances depend upon the solvent, the non planar distortion of the pyrrole rings (*ruffling*), and the π–π stacking phenomena in solvents like $CHCl_3$. Usually a bigger planarity deviation produces a red shift, while aggregations cause a blue shift.[14]

In our case, porphyrin **1**, bearing four aliphatic chains on the *meso* carbons, each one with a stereogenic centre at C-2 and a double bond at C-5, absorbed in $CHCl_3$ at 421 nm (*ruf*, see also the subsequent ^{1}H

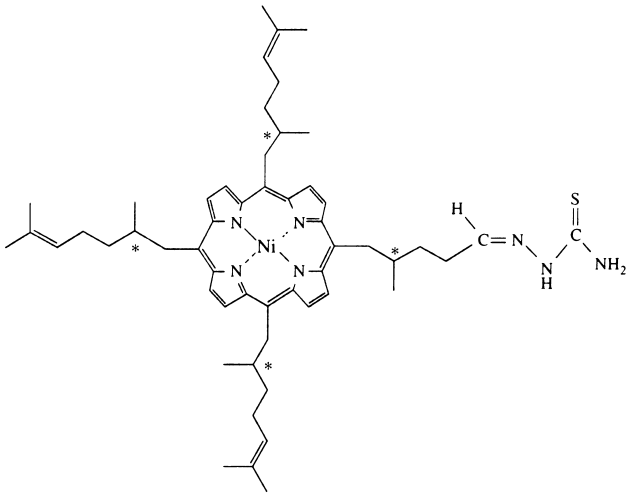


Figure 3. Nickel complex **13**

NMR spectroscopy paragraph), while macrocycle **2**, with two alkenylic chains and two *p*-fluoro-phenylic residues in *meso*, in $CHCl_3$ showed the Soret band at 414 nm (*wave*) (Table 1).

The introduction of the hydroxylic groups on the *meso* substituents produced a shift in the characteristic Soret band: this shift was usually a blue shift (major energy), with differences of a few nm from the starting porphyrin (Table 1). In fact the hydroxylated macrocycles (**4**–**8**), derived from compound **1**, all showed a blue shift with respect to **1**, probably due to the presence of inter- or intra-molecular hydrogen bonds involving the hydroxyls of the side chains and the electron-rich porphyrinic cavity. However, a different behaviour was observed inside this group of compounds (**4**–**8**) (Table 1).

The hydroxylated porphyrins **4**–**6** showed, in $CHCl_3$, a gradual red shift of the Soret band on increasing the number of the hydroxyls in the *meso* substituents: compound **4** (two hydroxyls) absorbed at 415 nm, **5** at 417 nm, while macrocycle **6** (four free hydroxyls) at 419 nm, showing shifts that are usually associated with the porphyrinic planarity deviation.[10,15]

It is remarkable that for compounds **7** and **8**, bearing six and eight hydroxyls, the Soret bands were found, in MeOH, at 410 and 414 nm, respectively. The blue shift was presumably caused by the simultaneous formation of hydrogen bonds and by disaggregation due to the MeOH solvent, that hindered the π–π stackings and coordinated the NH groups of the ring. Moreover all the Q bands were shifted to lower λ values with respect to the starting macrocycle **1**, while a decrease in the absorbance intensity was observed for all the hydroxyled porphyrins **4**–**8**.

Porphyrinic macrocycles **9**–**12**, which bear a different number of thiosemicarbazone residues in *meso* positions generated a new shift for the Soret band. Compounds **9**–**11** showed a red shift if compared to the initial hydroxylated compounds, explicable by the introduction of substituents which were on the whole planar but bulky, while the λ value did not vary significantly for product **12** (Table 1).

Porphyrins **9**–**12** exhibited the Soret band, respectively, at 419, 423, and 414 nm. Compound **9** (Soret band at 419 nm), derived from **4** (Soret band at 415 nm) and carrying just one thiosemicarbazonic chain in a *meso* position, presented a red shift of 4 nm. Porphyrins **10** and **11** behaved in the same way. The first one, **10**, with two thiosemicarbazidic residues, showed the Soret band at 423 nm, red shifted 4 nm with respect to the starting porphyrin **6**; the second one (**11**), bearing three thiosemicarbazidic chains in *meso* positions and coming from **7** (Soret band at 410 nm), absorbed at 414 nm. On the contrary, no significant shift was found for macrocycle **12** (with four thiosemicarbazonic groups), where the Soret band remained at 414 nm as in the original porphyrin **8**.

Table 1
UV–vis data (λ, nm; ε, cm^{-1} M^{-1}) of the synthesized porphyrins **1**–**13**

Porphyrin	Soret	Q_1	Q_2	Q_3	Q_4
1	421	522	558	602	660
	(ε = 47771)	(ε = 1831)	(ε = 1406)	(ε = 647)	(ε = 825)
2	414	514	549	590	646
	(ε = 25038)	(ε = 2401)	(ε = 1544)	(ε = 1239)	(ε = 883)
4	415	521	557	600	658
	(ε = 6433)	(ε = 504)	(ε = 350)	(ε = 168)	(ε = 226)
5	417	520	557	601	658
	(ε = 15820)	(ε = 968)	(ε = 741)	(ε = 373)	(ε = 420)
6	419	520	556	600	658
	(ε = 6359)	(ε = 357)	(ε = 251)	(ε = 141)	(ε = 158)
7	410	518	553	598	655
	(ε = 10407)	(ε = 885)	(ε = 596)	(ε = 302)	(ε = 333)
8	414	517	553	598	655
	(ε = 19345)	(ε = 1357)	(ε = 931)	(ε = 549)	(ε = 505)
9	419	519	555	598	657
	(ε = 4477)	(ε = 413)	(ε = 306)	(ε = 216)	(ε = 178)
10[a]	423	517	559	592	606
	(ε = 5010)	(ε = 224)	(ε = 224)	(ε = 189)	(ε = 174)
11	414	-	544	-	-
	(ε = 6315)		(ε = 330)		
12[b]	414	516	552	600	654
	(ε = 6639)	(ε = 724)	(ε = 527)	(ε = 359)	(ε = 282)
13	414	-	-	-	651
	(ε = 2450)				(ε = 123)

[a]Another weak Q band appeared (λ, 665 nm, ε=105 $cm^{-1}M^{-1}$).
[b]Another Q band was present (λ, 570 nm, ε=414 $cm^{-1}M^{-1}$)

For compounds **9**–**11** the functionalization to thiosemicarbazones could produce planarity deviations (*ruffling*),[15] that are clearly evident from the UV–vis bathochromic shifts of the Soret bands (419, 423 nm), and from the ^{1}H NMR NH chemical shifts (−2.09, −2.7 ppm),[10] and that are probably due to presence of several bulky substituents in the *meso* side chains. The experimental behaviour of porphyrin **12** could be explained considering that the symmetry of the macrocycle **12**, bearing four equal substituents in *meso* positions, remained the same as that found in the starting compound **8**, carrying eight hydroxyls on the side chains. Moreover, as previously noticed for the hydroxylated porphyrins **4**–**8**, even products **9**–**12** showed a decrease in the absorbance intensity, particularly remarkable for compound **11** where some Q bands disappeared.

Finally, as far as the only example studied of metallated porphyrinato was concerned, the nickel(II) porphyrinato **13**, derived from macrocycle **9**, absorbed at 414 nm and was 5 nm blue shifted with respect to the starting compound **9**. It is noticeable that the first three Q bands disappeared, while the Q_4 band was

Table 2
^{1}H NMR (400 MHz) chemical shifts of compounds **1** and **2**

Compound	H-β	H-5	H-1α	H-1β	H-4α	H-2	H-4β	NH
1	9.48 (8H, s)	5.10 (4H, m)	5.06 (4H, dd)	4.69 (4H, dd)	2.62 (4H, m)	2.32 (4H, m)	2.15 (4H, m)	- 2.65 (2H, bs)
2	9.50 (d) 8.91 (d) 8.83 (s) 8.78 (s)	4.89 (2H, m)	7.68 (2H, m)	7.52 (2H, m)	4.31 (2H, m)	4.21 (2H, m)	4.04 (2H, m)	- 1.50 (2H, bs)

present at 651 nm. The introduction of the nickel ion, whose preferential coordination is usually square planar, in the porphyrinic cavity produced a minor planarity deviation, as confirmed by the UV–vis blue shift of the Soret band (414 nm versus 419 nm in the free macrocycle **9**, Table 1), and further by the ^{1}H NMR slight shifts to low fields of all the protons in the spectrum (Table 4).

2.7. ^{1}H NMR spectroscopy

The up and down stereodisposition of the *meso* substituents with respect to the mean molecular plane (atropisomers)[16] and the nonplanar distortion of the pyrrole rings (*ruffling*)[15] were also investigated by means of ^{1}H NMR spectroscopy. In our case the atropisomers were due to the hindered rotation around the bond between the *meso* carbon of the porphyrin and the alkenyl substituent, to give preferentially compounds presenting a conformation αβαβ (e.g. compound **1**).

The ^{1}H NMR spectra of the synthesized alkenylporphyrins (**1** and **2**) were rather complicated, but showed ranges characteristic for the identification of the products. The monodimensional ^{1}H NMR (400 MHz) experiment for compound **1**, where the *meso* moieties are alternatively up and down with respect to the mean molecular plane, exhibited at room temperature one signal for all the eight aromatic protons H-β at 9.48 ppm (Table 2). This situation of high symmetry was not real, as further evidenced by the UV–vis spectroscopy (Soret band at 421 nm), but it was probably caused by the presence of two *ruffle* conformations (*ruf*) in fast equilibrium at room temperature, that should be seen at low tempearture when the chemical exchange becomes slow on the NMR time scale, as found in other reports.[13,15] The four H-5 hydrogens on the double bonds of the alkenylic chains showed a multiplet at 5.10 ppm (four protons), and the two NH groups inside the porphyrinic core shifted towards high fields beyond TMS giving one broad singlet at −2.65 ppm.

On the contrary, porphyrin **2** showed a complicated aromatic system consisting of two doublets (each integrating for two protons) and of two singlets (each integrating for two protons) (Table 2). The particular symmetry of the aromatic pattern could be due to a *wave* conformation as reported in literature.[15] The H_a protons (deshielded because they are near to the phenylic residue) and H_b (near to the alkenylic chain) of two pyrrole rings each exhibited a doublet (2H), respectively, at 9.50 and at 8.91 ppm: they are not equivalent, since they have different chemical surroundings. For H_c and H_d protons two singlets (each integrating two protons) were observed, at 8.83 and 8.78 ppm respectively: H_c and H_d presented a slightly different chemical shift, probably because of similar surroundings. Dreiding models also confirmed that,

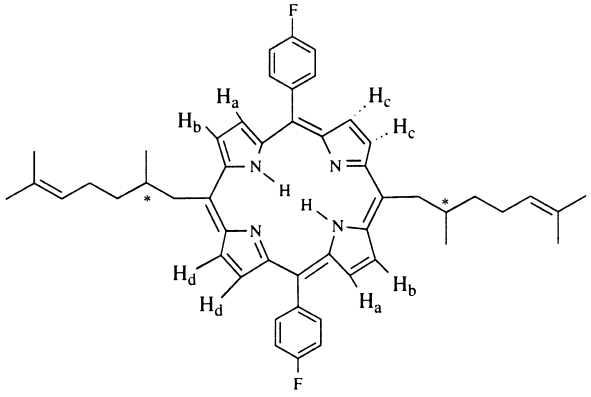


Figure 4. Protons H_c are below the mean porphyrin plane, while H_d are above

for a *wave* conformation with side chains in *trans*, the H_c and H_d protons were much further from the *meso* substituents than protons H_a and H_b (Fig. 4). The *ortho* and *meta* phenyl protons in the *meso* positions were found, respectively, at 8.16 and 7.43 ppm in compound **2**. All of the hydrogens on the alkenylic chains (H-1α, H-1β, H-2, H-4α, H-4β, H-3α and H-3β) were shifted to low fields compared to porphyrin **1**, while the two protons H-5 on the double bonds showed a multiplet at 4.89 ppm (2H), and the NH presented a broad singlet (2H) at −1.50 ppm.

As shown by a ^{1}H NMR analysis of the hydroxylated porphyrins **4**–**8**, the H-β aromatic system was modified in comparison with the initial macrocycle (Table 3). All the ^{1}H NMR spectra of compounds **4**–**8** were quite complicated, but with characteristic common ranges. Porphyrin **8**, bearing eight free hydroxyls, produced a symmetric aromatic system, consisting of one signal for the eight aromatic protons H-β at 7.62 ppm (Table 3). This symmetry was probably due to the fast interconversion between two *ruffle* conformations (*ruf*), that should be seen at a low temperature, as found for compound **1**.[13,15]

The thiosemicarbazonic derivates of porphyrins (**9**–**12**, Table 4) afforded ^{1}H NMR spectra that were very difficult to interpret, but characteristic common ranges with the main signals were found in this case too. Of course a major modification in the aromatic pattern was observed after the functionalization of the macrocycles to thiosemicarbazones (Table 4). In particular, for compounds **9**–**11** the NH signals of the porphyrinic ring shifted to low fields (−2.09, −2.7 ppm),[10] probably as a consequence of the introduction, on the *meso* side chains, of many electron donor bulky groups, that could produce shielding effects on the H-β protons (Table 4).

Finally, the nickel(II) porphyrinato **13**, deriving from macrocycle **9**, showed in the ^{1}H NMR experiment a slight shift in all the protons towards low fields (Table 4), to demonstrate the introduction of the metal ion into the porphyrinic cavity, also confirmed by the UV–vis blue shift of the Soret band (Table 1).

2.8. Biological tests: effects on cell proliferation and apoptosis induction

Compounds **1**, **6**, **9** and **10** have been tested in vitro on human leukemic cell lines U937, focusing our attention on their activity with respect to cell proliferation inhibition and apoptosis induction. Apoptosis is a form of self-regulated cell death that differs from necrosis with characteristic morphological changes that include membrane blebbing, chromatin condensation and the formation of apoptotic bodies. Apoptosis is necessary for normal embryonic and tissue differentiation. Cell death in tumours, whether spontaneous or treatment-induced, occurs predominantly via apoptosis rather than necrosis. It has been reported that a number of anticancer drugs may exert their activity by inducing apoptosis.[17] The

Table 3
^{1}H NMR (400 MHz) chemical shifts of the main hydroxylated porphyrins **4**, **6**, **7**, and **8** (**4** and **6** were dissolved in $CDCl_3$, **7** and **8** in CD_3OD)

Compound	H-β	OH	*CH*=C	H-1	*CH*OH	H-2+H-3	NH
4	9.4 (2H, bs), 7.68 and 7.51 (2H, 2d), 7.3-7.0 (4H, m)	5.31 (2H, bs)	5.1 (3H, m)	4.3 (8H, m)	4.2-3.4 (1H, m)	2.4-2.1 (12H, m)	- 2.67 (2H, bs)
6	9.43 (4H, bs), 7.32 (4H, bs)	5.8-5.61 (2H, 2 bs), 2.65 (2H, bs)	5.05 (2H, m)	4.7 (8H, m)	3.8-3.4 (2H, m)	2.5-2.0 (12H, m)	- 2.67, - 2.58 (2H, 2 bs)
7	9.53 (2H, bs), 7.58 (6H, s)	nr	5.08 (1H, m)	4.6-4.2 (8H, m)	3.9-3.5 (3H, m)	2.5-2.0 (12H, m)	nr
8	7.62 (8H, s)	nr	—	4.25 (8H, m)	3.8-3.5 (4H, m)	2.7-2.0 (12H, m)	nr

mechanisms underlying the effects vary depending on the initiating stimulus but a common feature is the activation of the endonuclease leading to DNA fragmentation.

All compounds (**1**, **6**, **9** and **10**) remarkably inhibited cell proliferation, in particular porphyrins **1** and **10** when used at 20 μg/ml, and compounds **6** and **9** at 10 μg/ml respectively (Fig. 5).

Using these macrocycles in the apoptosis assays only compound **1** was able to cause DNA fragmentation at these concentrations, inducing apoptosis (Fig. 6).

It means that these two biological processes (cell proliferation and apoptosis induction) present different mechanisms in their interactions with substrates: in fact porphyrin **1**, that bears four alkenylic chains on the *meso* carbons, induces apoptosis, while the superstructured macrocycles **9** and **10**, functionalized with thiosemicarbazonic residues in *meso*, possess an increased activity with regard to the inhibition of the cell proliferation.

Table 4
^{1}H NMR (400 MHz) chemical shifts of the thiosemicarbazonic derivatives of porphyrins **9**–**13**

Compound	H-β	NHCS	*CH*=C	*CH*=N	H-1	H-2+H-3	NH
9	7.71 and 7.55 (4H, 2s), 7.33 and 6.93 (2H, 2d), 7.1 (2H, m)	7.3 (1H, bs)	5.25 (3H, m)	4.67 (1H, t)	4.3 and 4.09 (8H, 2m)	2.4-2.1 (12H, m)	- 2.09, - 2.91 (2H, 2 bs)
10	7.72 and 7.63 (8H, 2m)	10.12 and 10.05 (2H, 2 bs)	5.77 (2H, m)	6.15 (2H, m)	5.58 and 4.19 (8H, 2m)	2.3-1.8 (12H, m)	- 2.7, (2H, bs)
11	8.51 (2H, m), 8.03 and 7.21 (2H, 2m), 7.72 and 7.71 (2H, 2d), 7.61 and 7.59 (2H, 2d)	nr	5.87 (1H, m)	5.67 (3H, m)	5.58 and 5.38 (8H, 2m)	2.3-2.1 (12H, m)	nr
12	7.62 (2H, m), 7.73 and 7.55 (2H, 2m), 7.44 and 7.28 (2H, 2m), 7.36 (2H, m)	nr	—	5.33 (4H, t)	3.8-3.5 (8H, m)	2.23-2.21 (12H, m)	nr
13	7.65 and 7.53 (4H, 2s), 7.38 and 7.05 (2H, 2d), 7.15 (2H, m)	nr	5.27 (3H, m)	5.18 (1H, m)	4.45 (8H, m)	2.3-2.1 (12H, m)	—

3. Experimental

3.1. General

All solvents were distilled before use: Et_2O over $LiAlH_4$, CH_2Cl_2 over CaH_2. Pyrrole was from Acros, (3*R*)-(+)-citronellal from Fluka, *p*-fluorobenzaldehyde from Sigma and thiosemicarbazide from Janssen. Porphyrinic compounds were visualized as green-emerald spots by dipping the plates in a solution of Ce(III)sulfate (1.0 g), ammonium molybdate (21 g), 96% sulfuric acid (31 ml), and distilled water (500 ml). TLC was performed on E. Merck plates precoated with SiO_2 F_{254}. Flash chromatography was performed on a Carlo Erba Florisil (100–200 mesh). ^{1}H NMR were obtained on a Brucker AMX-400 spectrometer, and chemical shifts are given in units of δ relative to TMS as an internal reference. Optical rotations were measured on a Rudolph Autopol III polarimeter. UV–vis were measured on a Kontron

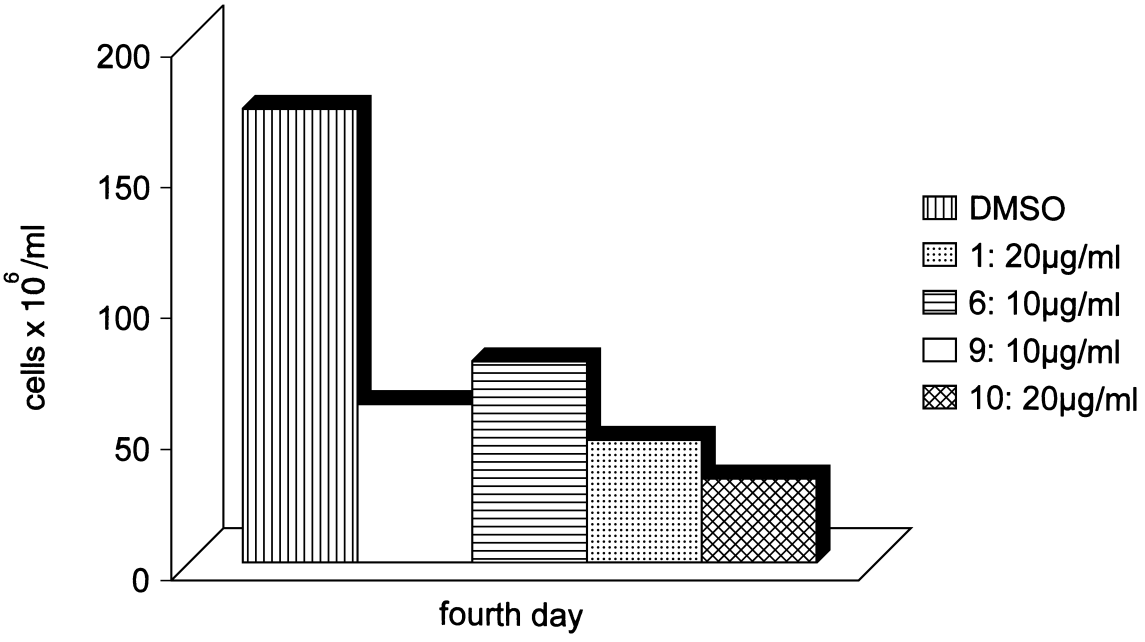


Figure 5.

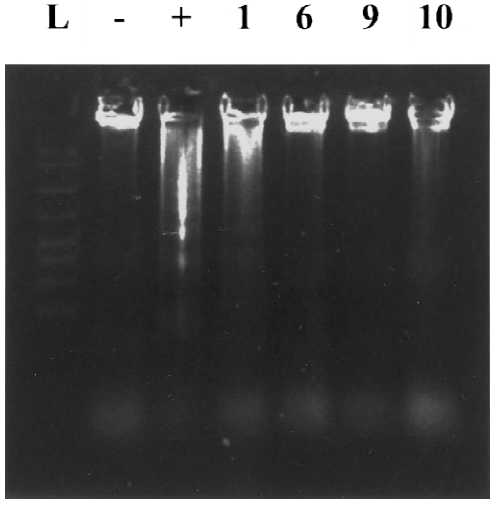


Figure 6. Apoptosis assays on compounds **1**, **6**, **9** and **10**: lane L ladder; lane − untreated control cells; lane + positive control; lane 1 compound **1**; lane 2 compound **6**; lane 3 compound **9**; lane 4 compound **10**

Uvicon 860 spectrophotometer. CI-MS (m/z, 70 eV) data were obtained on a Finnegan 1020 6c mass spectrometer. Elemental analyses were performed by the Microanalytical Laboratory of the University of Parma (Dipartimento di Chimica Generale ed Inorganica).

*3.2. Synthesis of alkenylporphyrins **1** and **2***

*3.2.1. 5α,10β,15α,20β-Tetrakis-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrin **1***

To a solution of $Mg_{(s)}$ (240 mg, 10 mmol) and EtBr (3 ml), in dry Et_2O (30 ml), was added pyrrole (0.7 ml, 10 mmol), under a stream of argon. Then, after concentrating under vacuum, anhydrous CH_2Cl_2

(100 ml), and (3*R*)-(+)-citronellal (1 ml, 5 mmol, in a molar ratio 1:2 with respect to pyrrolylmagnesiumbromide) were added, under argon. The reaction vessel was carefully shielded from light, and stirring was continued for 4 h. Then the reaction was quenched by addition of a saturated NH_4Cl solution, the organic phase was extracted with Et_2O and dried over $MgSO_4$. Subsequently the filtered solution was oxidized for 48 h in air, and the resulting red dark-violet solid was purified by elution with hexane:ethylacetate (90:10), affording compound **1** as a blue-fluorescent red brown powder (7.8% yield). R_f 0.66 (hexane:ethylacetate, 90:10). $[\alpha]_{546}^{20}$ −309.5; $[\alpha]_{589}^{20}$ −166.7 (*c*=0.84, $CHCl_3$). UV–vis ($CHCl_3$) λ_{max} 421 nm (ε=47771 cm^{-1} M^{-1}), 522 (ε=1831), 558 (ε=1406), 602 (ε=647), 660 (ε=825). ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* in hertz) 9.48 (8H, s, H-β), 5.10 (4H, m, H-5), 5.06 (4H, dd, *J*=14.3, *J*=6.4, H-1α), 4.69 (4H, dd, *J*=14.6, *J*=8.5, H-1β), 2.62 (4H, m, H-4α), 2.32 (4H, m, H-2), 2.15 (4H, m, H-4β), 1.78 (4H, m, H-3α), 1.71 (4H, m, H-3β), 1.69 and 1.61 (24H, 2s, 12H each, *CH*$_3$C=C), 1.02 (12H, d, *J*=6.8, CH*CH*$_3$), −2.65 (2H, bs, NH). MS (CI, CH_4) 807 (M+1). Anal. calcd for $C_{56}H_{78}N_4$: C 83.32, H 9.74, N 6.94; found: C 83.45, H 9.88, N 6.67.

*3.2.2. 5,15-Di-[*p*-fluoro-phenyl]-10α,20β-di-[(2*R*)-2,6-dimethyl-5-eptenyl]-porphyrin **2***

The known compound 4′-fluoro-phenyldipyrrylmethane[5] was salified to 4′-fluoro-phenyldipyrrylmethanebismagnesiumbromide **3** using EtMgBr (2:1 molar ratio compared to the dimeric compound) in dry Et_2O (30 ml); the solvent was subsequently removed under vacuum, and anhydrous CH_2Cl_2 (90 ml) was added. Compound **3** (0.4 mmol) gave, in the presence of (3*R*)-(+)-citronellal (0.07 ml, 0.4 mmol), after 4 h under argon, the corresponding porphyrinogens (not isolated), then the reaction mixture was treated with dichlorodicyanobenzoquinone (DDQ) and followed by NH_4Cl solution quenching. The organic phase was extracted with CH_2Cl_2, dried on $MgSO_4$ and purified with hexane:ethylacetate, (90:10) affording the porphyrinic macrocycle **2** as a blue-fluorescent red brown powder (4.7% yield). R_f 0.47 (hexane:ethylacetate, 90:10). $[\alpha]_{546}^{20}$ −238.2; $[\alpha]_{589}^{20}$ −64.8 (*c*=0.11, $CHCl_3$). UV–vis ($CHCl_3$) λ_{max} 414 nm (ε=25038 cm^{-1} M^{-1}), 514 (ε=2401), 549 (ε=1544), 590 (ε=1239), 646 (ε=883). ^1H NMR (400 MHz, $CDCl_3$) δ (multiplicity, *J* in hertz) 9.50 (2H, d, *J*=5.0, H-β), 8.91 (2H, d, *J*=5.0, H-β), 8.83 (2H, s, H-β), 8.78 (2H, s, H-β), 8.16 (4H, m, H *ortho* phenyl), 7.68 (2H, m, H-1α), 7.52 (2H, m, H-1β), 7.43 (4H, m, H *meta* phenyl), 4.89 (2H, m, H-5), 4.31 (2H, m, H-4α), 4.21 (2H, m, H-2), 4.04 (2H, m, H-4β), 3.62 (4H, m, H-3α+H-3β), 1.82 and 1.62 (12H, 2s, 6H each, *CH*$_3$C=C), 1.33 (6H, d, *J*=6.8, CH*CH*$_3$), −1.50 (2H, bs, NH). MS (CI, CH_4) 747 (M+1). Anal. calcd for $C_{50}H_{52}N_4F_2$: C 80.40, H 7.02, N 7.50; found: C 80.56, H 7.14, N 7.38.

*3.3. Synthesis of oxydrylated porphyrins **4–8***

To a solution of porphyrin **1** (20.15 mg, 0.025 mmol), in CH_2Cl_2 dry (5 ml), *cis*-dicyclo-hexane-18-crown-6 ether (1.86 mg, 0.005 mmol) and powdered $KMnO_4$ (2.38 mg, 0.015 mmol) were added at 263 K, under a stream of argon.[6] After stirring for 5 h, the reaction was quenched by addition of a saturated Na_2SO_3 solution and a 5% citric acid solution. The mixture was extracted with CH_2Cl_2 (2×20 ml), dried on $MgSO_4$, filtered and evaporated under vacuum. Elution with CH_2Cl_2:isopropanol (99:1) allowed the separation of pure porphyrins **4** (12%), **5** (8%), **6** (22%), **7** (23%) and **8** (30%) as blue-fluorescent red-brown powders.

*3.3.1. 5α-[(2*R*)-2,6-Dimethyl-5,6-dihydroxyeptyl]-10β,15α,20β-tris-[(2*R*)-2,6-dimethyl-5-eptenyl]-porphyrin **4***

R_f 0.78 (CH_2Cl_2:isopropanol, 99:1). UV–vis ($CHCl_3$) λ_{max} 280 nm (ε=555 cm^{-1} M^{-1}), 415 (ε=6433), 521 (ε=504), 557 (ε=350), 600 (ε=168), 658 (ε=226). ^1H NMR (400 MHz, $CDCl_3$) δ 9.40 (2H, bs, H-β),

7.68 (1H, d, *J*=5.0, H-β), 7.51 (1H, d, *J*=5.0, H-β), 7.3–7.0 (4H, m, H-β), 5.31 (2H, bs, OH), 5.1 (3H, m, *CH*=C(CH_3)$_2$ in C-5), 4.3 (8H, m, H-1), 4.2–3.4 (9H, 1H+8H, m, *CH*OH in C-5+H-4), 2.4–2.1 (12H, 4H+8H, m, H-2+H-3), 2.0–0.8 (36H, m, 6 *CH*$_3$C=C+4 CH*CH*$_3$ +2 *CH*$_3$COH), −2.67 (2H, bs, NH). MS (CI, CH_4) 841 (M+1). Anal. calcd for $C_{56}H_{80}N_4O_2$: C 79.94, H 9.59, N 6.66; found C 80.01, H 9.64, N 6.57.

*3.3.2. 5α,10β-Di-[(2R)-2,6-dimethyl-5,6-dihydroxyeptyl]-15α,20β-di-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrin **5***

R_f 0.72 (CH_2Cl_2:isopropanol, 99:1). UV–vis ($CHCl_3$) λ_{max} 286 nm (ε=629 cm^{-1} M^{-1}), 417 (ε=15820), 520 (ε=968), 557 (ε=741), 601 (ε=373), 658 (ε=420). ^{1}H NMR (400 MHz, $CDCl_3$) spectrum is difficult to discuss even if the signals appear in the same ranges as found for the oxydrylated compounds **4**–**8**. MS (CI, CH_4) 875 (M+1). Anal. calcd for $C_{56}H_{82}N_4O_4$: C 76.83, H 9.45, N 6.40; found: C 76.95, H 9.52, N 6.26.

*3.3.3. 5α,15α-Di-[(2R)-2,6-dimethyl-5,6-dihydroxyeptyl]-10β,20β-di-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrin **6***

R_f 0.45 (CH_2Cl_2:isopropanol, 99:1). UV–vis ($CHCl_3$) λ_{max} 281 (ε=127 cm^{-1} M^{-1}), 419 (ε=6359), 520 (ε=357), 556 (ε=251), 600 (ε=141), 658 (ε=158). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (4H, bs, H-β), 7.32 (4H, bs, H-β), 5.8–5.61 (2H, 2 bs, OH), 5.05 (2H, m, *CH*=C(CH_3)$_2$ in C-5), 4.7 (8H, m, H-1), 3.8–3.4 (10H, 2H+8H, m, *CH*OH in C-5+H-4), 2.65 (2H, bs, OH), 2.5–2.0 (12H, 4H+8H, m, H-2+H-3), 1.86 (12H, s, *CH*$_3$C=C), 1.58 (12H, d, *J*=7.0, CH*CH*$_3$), 1.26 (12H, s, *CH*$_3$COH), −2.67 and −2.58 (2H, 2 bs, NH). MS (CI, CH_4) 875 (M+1). Anal. calcd for $C_{56}H_{82}N_4O_4$: C 76.83, H 9.45, N 6.4; found: C 76.9, H 9.5, N 6.32.

*3.3.4. 5α,10β,15α-Tris-[(2R)-2,6-dimethyl-5,6-dihydroxyeptyl]-20β-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrin **7***

R_f 0.16 (CH_2Cl_2:isopropanol, 99:1). UV–vis (MeOH) λ_{max} 282 (ε=619 cm^{-1} M^{-1}), 285 (ε=635), 410 (ε=10407), 518 (ε=885), 553 (ε=596), 598 (ε=302), 655 (ε=333). ^{1}H NMR (400 MHz, CD_3OD) δ 9.53 (2H, bs, H-β), 7.58 (6H, s, H-β), 5.08 (1H, m, *CH*=C(CH_3)$_2$ in C-5), 4.6–4.2 (8H, m, H-1), 3.9–3.5 (11H, 3H+8H, m, *CH*OH in C-5+H-4), 2.5–2.0 (12H, 4H+8H, m, H-2+H-3), 1.85 (6H, s, *CH*$_3$C=C), 1.55 (18H, s, *CH*$_3$COH), 1.18 (12H, m, CH*CH*$_3$). MS (CI, CH_4) 909 (M+1). Anal. calcd for $C_{56}H_{84}N_4O_6$: C 73.96, H 9.32, N 6.16; found: C 73.92, H 9.38, N 6.10.

*3.3.5. 5α,10β,15α,20β-Tetrakis-[(2R)-2,6-dimethyl-5,6-dihydroxyeptyl]-porphyrin **8***

R_f 0.10 (CH_2Cl_2:isopropanol, 99:1). UV–vis (MeOH) λ_{max} 287 (ε=1480 cm^{-1} M^{-1}), 290 (ε=1435), 414 (ε=19345), 517 (ε=1357), 553 (ε=931), 598 (ε=549), 655 (ε=505). ^{1}H NMR (400 MHz, CD_3OD) δ 7.62 (8H, s, H-β), 4.25 (8H, m, H-1), 3.8–3.5 (12H, 4H+8H, m, *CH*OH in C-5+H-4), 2.7–2.0 (12H, 4H+8H, m, H-2+H-3), 1.7–1.1 (36H, 24H+12H, m, 8 *CH*$_3$COH+4 CH*CH*$_3$). MS (CI, CH_4) 943 (M+1). Anal. calcd for $C_{56}H_{86}N_4O_8$: C 71.29, H 9.19, N 5.94; found: C 71.25, H 9.25, N 5.87.

*3.4. Synthesis of the thiosemicarbazonic derivates of porphyrins **9**–**13***

*3.4.1. 5α-[(2R)-2-Methyl-5-pentancarboxyaldehydethiosemicarbazonyl]-10β,15α,20β-tris-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrin **9***

To an aqueous solution of $NaIO_4$ (0.84 mg, 0.004 mmol) were added SiO_2 (6 mg, 0.1 mmol) and CH_2Cl_2 (20 ml), with stirring at room temperature. Then the red solution of porphyrin **4** (2.5 mg, 0.003

mmol) in CH_2Cl_2 (3 ml) was poured into the reaction vessel. After 1 h the reaction mixture was filtered on a Buchner, dried on dry Na_2SO_4, filtered and evaporated to give the corresponding aldehyde **14** as a green powder (1.37 mg, 1.76 μmol, 55% yield). Meanwhile the thiosemicarbazide (0.16 mg, 1.76 μmol, molar ratio 1:1 with respect to porphyrin **14**) was dissolved, with stirring, in EtOH 95% (5 ml) and subsequently added to the solution of the porphyrin dissolved in EtOH 95% (3 ml). After 1 h the reaction mixture was filtered to afford the thiosemicarbazonic derivate **9** as a bright yellow powder (1.34 mg, 1.57 μmol, 98% yield). $[\alpha]^{20}_{546}$ +106.5; $[\alpha]^{20}_{589}$ +135.8 (*c*=0.048, MeOH). UV–vis ($CHCl_3$) λ_{max} 283 nm (ε=3563 cm^{-1} M^{-1}), 288 (ε=3390), 297 (ε=1596), 419 (ε=4477), 519 (ε=413), 555 (ε=306), 598 (ε=216), 657 (ε=178). ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (2H, s, H-β), 7.55 (2H, s, H-β), 7.33 (1H, d, *J*=5.0, H-β), 7.3 (1H, bs, NHCS), 7.1 (2H, m, H-β), 6.93 (1H, d, *J*=5.0, H-β), 5.25 (3H, m, *CH*=C$(CH_3)_2$ in C-5), 4.67 (1H, t, *J*=6.5, *CH*=N in C-5), 4.3 (4H, m, H-1α), 4.09 (8H, m, H-1β+H-4α), 3.65 (6H, 4H+2H, m, H-4β+NH_2), 2.4–2.1 (12H, 4H+8H, m, H-2+H-3), 2.0–0.8 (30H, m, 6 *CH*$_3$C=C+4 CH*CH*$_3$), −2.09 and −2.91 (2H, 2 bs, NH). MS (CI, CH_4) 854 (M+1). Anal. calcd for $C_{54}H_{75}N_7S$: C 75.92, H 8.86, N 11.48, S 3.75; found: C 75.89, H 8.94, N 11.41, S 3.77.

*3.4.2. 5α,10α-Di-[(2R)-2-methyl-5-pentancarboxyaldehydethiosemicarbazonyl]-10β,20β-di-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrin **10***

To an aqueous solution of $NaIO_4$ (2.78 mg, 0.013 mmol) were added SiO_2 (20 mg, 0.333 mmol) and CH_2Cl_2 (20 ml), under stirring and at room temperature. Then the red solution of porphyrin **6** (4.37 mg, 0.005 mmol) in CH_2Cl_2 (3 ml) was poured into the reaction vessel. After 1 h the reaction mixture was filtered on a Buchner, dried on dry Na_2SO_4, filtered and evaporated to give the corresponding aldehyde **15** as a red powder (2.4 mg, 3.18 μmol, 55% yield). Meanwhile the thiosemicarbazide (0.58 mg, 6.37 μmol, molar ratio 2:1 with respect to porphyrin **15**) was dissolved, under stirring, in EtOH 95% (5 ml) and subsequently added to the solution of the porphyrin dissoved in EtOH 95% (3 ml). After 1 h the reaction mixture was filtered to afford the thiosemicarbazonic derivate **10** as a pale green powder (2.26 mg, 2.51 μmol, 94% yield). $[\alpha]^{20}_{546}$ +19.6; $[\alpha]^{20}_{589}$ +41.1 (*c*=0.107, MeOH). UV–vis (MeOH) λ_{max} 286 nm (ε=1627 cm^{-1} M^{-1}), 289 (ε=3254), 294 (ε=3443), 423 (ε=5010), 517 (ε=224), 559 (ε=224), 592 (ε=189), 606 (ε=174), 665 (ε=105). ^1H NMR (400 MHz, CD_3COCD_3) δ 10.12 and 10.05 (2H, 2 bs, NHCS), 7.72 and 7.63 (8H, 2m, 4H each, H-β), 6.15 (2H, m, *CH*=N in C-5), 5.77 (2H, m, *CH*=C$(CH_3)_2$ in C-5), 5.58 (2H, m, H-1α), 4.19 (6H, 2H+4H, m, H-1α+H-1β), 4.04 (6H, 4H+2H, m, H-4α+H-4β), 3.61 (6H, 2H+4H, m, H-4β+NH_2), 2.3–1.8 (12H, 4H +8H, m, H-2+H-3), 1.8–1.2 (24H, m, 4 *CH*$_3$C=C+4 CH*CH*$_3$), −2.7 (2H, bs, NH). MS (CI, CH_4) 901 (M+1). Anal. calcd for $C_{52}H_{72}N_{10}S_2$: C 69.29, H 8.06, N 15.55, S 7.1; found: C 69.25, H 8.12, N 15.48, S 7.15.

*3.4.3. 5α,10β,15α-Tris-[(2R)-2-methyl-5-pentancarboxyaldehydethiosemicarbazonyl]-20β-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrin **11***

To an aqueous solution of $NaIO_4$ (4.17 mg, 0.0195 mmol) were added SiO_2 (30 mg, 0.5 mmol) and CH_2Cl_2 (20 ml), under stirring and at room temperature. Then the red solution of porphyrin **7** (4.54 mg, 0.005 mmol) in MeOH (4 ml) was poured into the reaction vessel. After 1 h the reaction mixture was filtered on a Buchner, dried on dry Na_2SO_4, filtered and evaporated to give the corresponding aldehyde **16** as a bright yellow powder (2.54 mg, 3.49 μmol, 56% yield). Meanwhile the thiosemicarbazide (0.95 mg, 10.48 μmol, molar ratio 3:1 with respect to porphyrin **16**) was dissolved, under stirring, in MeOH (4 ml) and subsequently added to the solution of the porphyrin dissolved in MeOH (4 ml). After 1 h the reaction mixture was filtered to afford the thiosemicarbazonic derivate **11** as a yellow green powder (2.31 mg, 2.44 μmol, 91% yield). $[\alpha]^{20}_{546}$ +14.3; $[\alpha]^{20}_{589}$ +28.6 (*c*=0.035, MeOH). UV–vis (MeOH) λ_{max} 280 (ε=3074 cm^{-1} M^{-1}), 287 (ε=2154), 290 (ε=1826), 414 (ε=6315), 544 (ε=330). ^1H NMR (400 MHz,

CD_3OD) δ 8.51 (2H, m, H-β), 8.03 (1H, m, H-β), 7.72 and 7.71 (2H, 2d, *J*=4.8, H-β), 7.61 and 7.59 (2H, 2d, *J*=4.8, H-β), 7.21 (1H, m, H-β), 5.87 (1H, m, *CH*=C$(CH_3)_2$ in C-5), 5.67 (3H, m, *CH*=N in C-5), 5.58 (4H, m, H-1α), 5.38 (4H, m, H-1β), 3.7–3.5 (8H, m, H-4), 2.3–2.1 (12H, 4H+8H, m, H-2+H-3), 1.92 (12H, d, *J*=7.0, 4 CH*CH*$_3$), 1.33 (6H, m, 2 *CH*$_3$C=C). MS (CI, CH_4) 948 (M+1). Anal. calcd for $C_{50}H_{69}N_{13}S_3$: C 63.32, H 7.34, N 19.21, S 10.12; found: C 63.26, H 7.42, N 19.17, S 10.14.

*3.4.4. 5α,10β,15α,20β-Tetrakis-[(2R)-methyl-5-pentancarboxyaldehydethiosemicarbazonyl]-porphyrin **12***

To an aqueous solution of $NaIO_4$ (7.08 mg, 0.033 mmol) were added SiO_2 (51 mg, 0.848 mmol) and CH_2Cl_2 (20 ml), under stirring and at room temperature. Then the red solution of porphyrin **8** (6 mg, 6.37 μmol) in MeOH (4 ml) was poured into the reaction vessel. After 1 h the reaction mixture was filtered on a Buchner, dried on dry Na_2SO_4, filtered and evaporated to give the corresponding aldehyde **17** as a yellow powder (3.42 mg, 4.87 μmol, 57% yield). Meanwhile the thiosemicarbazide (1.78 mg, 19.49 μmol, molar ratio 4:1 with respect to porphyrin **17**) was dissolved, under stirring, in MeOH (4 ml) and subsequently added to the solution of the porphyrin dissolved in MeOH (4 ml). After 1 h the reaction mixture was filtered to afford the thiosemicarbazonic derivate **12** as a pale yellow powder (3.11 mg, 3.13 μmol, 91% yield). $[\alpha]^{20}_{546}$ +10.8; $[\alpha]^{20}_{589}$ +15.0 (*c*=0.167, MeOH). UV–vis (MeOH) λ_{max} 280 (ε=1623 cm^{-1} M^{-1}), 297 (ε=1623), 301 (ε=1623), 414 (ε=6639), 516 (ε=724), 552 (ε=527), 570 (ε=414), 600 (ε=359), 654 (ε=282). ^{1}H NMR (400 MHz, CD_3OD) δ 7.73 (1H, m, H-β), 7.62 (2H, m, H-β), 7.55 (1H, m, H-β), 7.44 (1H, m, H-β), 7.36 (2H, m, H-β), 7.28 (1H, m, H-β), 5.33 (4H, t, *J*=5.0, *CH*=N in C-5), 3.8–3.5 (16H, m, H-1+H-4), 2.23–2.21 (12H, 4H+8H, m, H-2+H-3), 2.05–1.8 (12H, m, 4 CH*CH*$_3$). MS (CI, CH_4) 995 (M+1). Anal. calcd for $C_{48}H_{66}N_{16}S_4$: C 57.92, H 6.69, N 22.53, S 12.86; found: C 57.89, H 6.76, N 22.48, S 12.87.

*3.4.5. 5α-[(2R)-2-Methyl-5-pentancarboxyaldehydethiosemicarbazonyl]-10β,15α,20β-tris-[(2R)-2,6-dimethyl-5-eptenyl]-porphyrinatonickel(II) **13***

To a solution of porphyrin **9** (1.34 mg, 1.57 μmol) in $CHCl_3$:CH_3OH 1:1 (10 ml), following Buchler's procedure,[12] $Ni(Ac)_2 \cdot 4H_2O$ (0.43 mg, 1.73 μmol) was added at room temperature under stirring. The mixture was sonicated on TLC until the green-emerald spot of the starting porphyrin had disappeared (3 h). The solvent was evaporated under vacuum to give, after TLC purification, the yellow green powder **13** (1.21 mg, 1.33 μmol, 90%). $[\alpha]^{20}_{546}$ +34.8; $[\alpha]^{20}_{589}$ +43.1 (*c*=0.06, MeOH). UV–vis (MeOH) λ_{max} 283 (ε=1886 cm^{-1} M^{-1}), 288 (ε=1367), 290 (ε=628), 414 (ε=2450), 651 (ε=123). ^{1}H NMR (400 MHz, CD_3OD) δ 7.65 (2H, s, H-β), 7.53 (2H, s, H-β), 7.38 (1H, d, *J*=4.8, H-β), 7.15 (2H, m, H-β), 7.05 (1H, d, *J*=4.8, H-β), 5.27 (3H, m, *CH*=C$(CH_3)_2$ in C-5), 5.18 (1H, m, *CH*=N in C-5), 4.45 (8H, m, H-1), 4.21 (4H, m, H-4α), 3.96 (4H, m, H-4β), 2.3–2.1 (12H, 4H+8H, m, H-2+H-3), 1.3–1.1 (18H, m, 6 *CH*$_3$C=C), 0.9 (12H, d, *J*=7.0, 4 CH*CH*$_3$). Anal. calcd for $C_{54}H_{73}NiN_7S$: C 71.25, H 8.09, N 10.78, S 3.52; found: C 71.19, H 8.14, N 10.71, S 3.55.

3.5. Biological data: materials and methods

The cells U937 were seeded at 2×10^5/ml in the presence of RPMI 1640 supplemented with L-glutamine 200 mM, 10% fetal bovine serum (FBS) and with antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml), and then treated with porphyrins **1** and **10** at 20 μg/ml, and with compounds **6** and **9** at 10 μg/ml. All compounds were previously stored dry at room temperature and dissolved in dimethyl sulphoxide (DMSO) just before their use. Cell mortality, evaluated on the fourth day by the trypan blue exclusion method and determined by using a hemocytometer, never exceeded 5%.

For the apoptosis assay the cells U937 were seeded at 5×10^5/ml in the presence of the above mentioned porphyrins at the same concentrations as used for the proliferation inhibition. After 18 h the cells (10^6) were washed twice with PBS at 2000 rpm for 10 min at 4°C, and then 20 μl of a lysis buffer solution (EDTA 10 mM, Tris HCl 50 mM pH 8.0 and 0.5% (w/v) sodium laurylsarkosinate) was added. The pellet was subsequently redissolved and 2.5 μl of Proteinase K was added (4 mg/ml). After 1 h at 50°C, 2.5 μg/ml of Rnase A (2 mg/ml) was added and incubated for 1 h at 40°C and then for 10 min at 70°C. 10 μl of loading buffer (EDTA 10 mM pH 8, 1% agarose, 0.25% bromophenol blue, 40% sucrose) were subsequently added to the extracted DNA, which was loaded on the agarose gel (2%) with 0.1 μg/ml ethidium bromide to evaluate the characteristic effects of apoptosis by means of electrophoresis.

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