Carboranyl-porphyrazines for anticancer therapies

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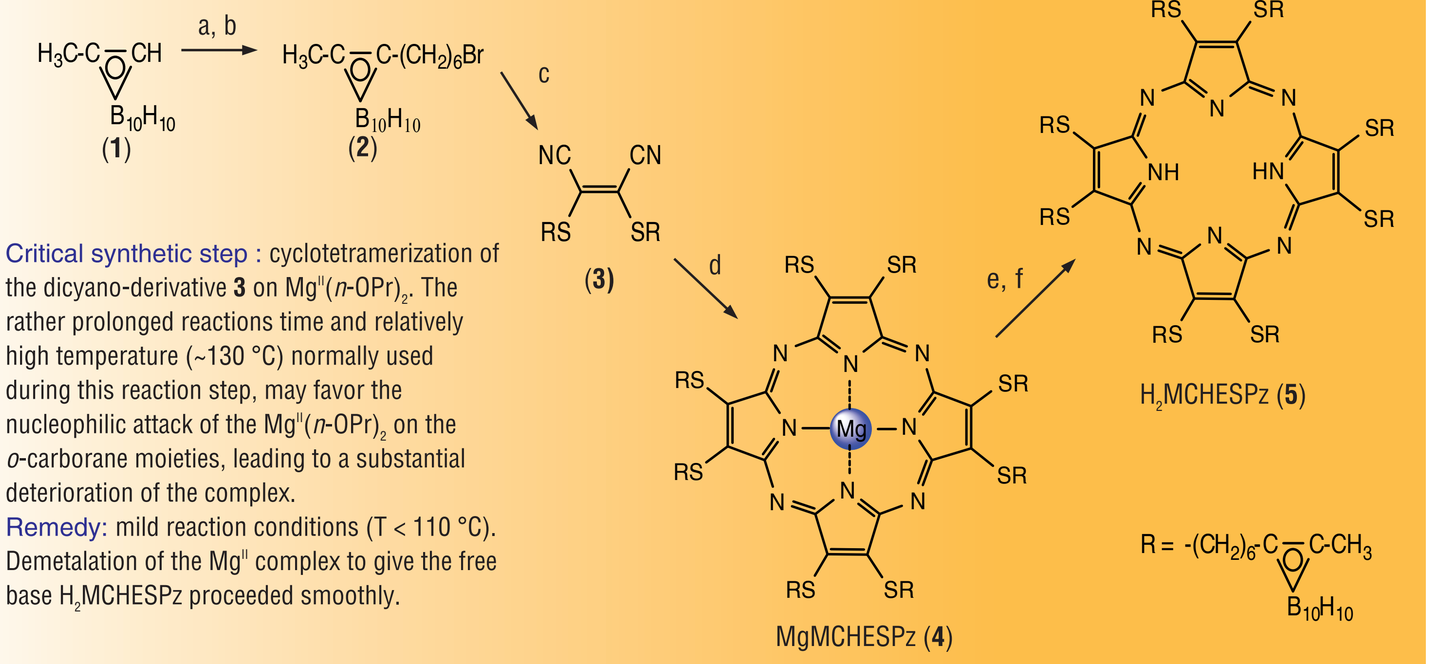
Highlight

In recent years we have focused our efforts on the synthesis and characterization of new families of alkylthioporphyrazines bearing highly boronated chemical functions for use in BNCT and multiple approach cancer therapy. The knowledge that carboranyl-porphyrazines may conjugate a high degree of peripheral carboranyl substitution with the peculiar electronic properties of the porphyrazinethiolate core – intense absorption in the "therapeutic window", near-IR luminescence, or rapid radiationless decay of the primarily excited $S_1(Q)$ state – motivated this choice. The possibility that these nanosized molecules can be effectively incorporated into liposomial vectors, was also explored successfully.[1] Here we report on the synthesis, characterization[2] and liposome insertion of novel carboranyl-porphyrazine derivatives, designed to improve the potentiality in multiple approach anticancer therapy. The obtained formulations were subsequently tested for BNCT efficacy on model cell lines (DHD/K12/TRb rat coloncarcinoma).[3]

Results and discussions

Synthesis of H_2MCHESPz

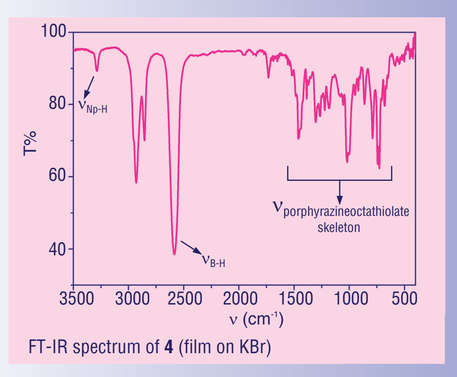
The synthesis of *closo*-carboranylporphyrazines is a multistep process involving template condensation of the pertinent carboranyl-maleonitrile derivative on $Mg^{II}(n\text{-OPr})_2$.



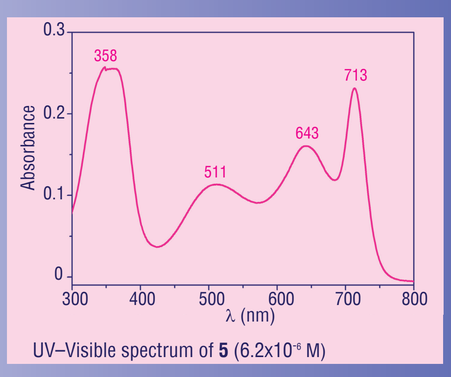
Critical synthetic step : cyclotetramerization of the dicyano-derivative **3** on $Mg^{II}(n\text{-OPr})_2$. The rather prolonged reactions time and relatively high temperature (~130 °C) normally used during this reaction step, may favor the nucleophilic attack of the $Mg^{II}(n\text{-OPr})_2$ on the *o*-carborane moieties, leading to a substantial deterioration of the complex.
Remedy: mild reaction conditions (T < 110 °C). Demetalation of the Mg^{II} complex to give the free base H_2MCHESPz proceeded smoothly.

Reagents and conditions: (a) *n*-BuLi, THF, under Ar, - 78 °C - r.t., 2 h; (b) $Br(CH_2)_6Br$, THF, under Ar, - 78 °C - r.t., 2 h; (c) $[dmmnt]Na_2$, MeOH/EtOH 8:2, 0 °C - r.t., in the dark, 36 h; (d) $Mg^{II}(n\text{-OPr})_2$, *n*-PrOH, reflux 110 °C, 24 h (e) TFA, $CHCl_3$; (f) NH_3, pH=7, on ice.

CHARACTERIZATION: The integrity of the *o*-carborane moieties in MgMCHESPz and H_2MCHESPz was confirmed by ^1H, ^{13}C, ^{10}B NMR, and IR spectra. For instance, the ^{13}C NMR spectrum of the Mg^{II} complex showed two resonances at δ = 78.2 ppm and δ = 74.9 ppm which are characteristic of the carbons of intact *closo*-carborane cages; the IR spectrum showed the symmetric strong peak at 2588 cm^{-1}, due to the B–H stretching of the *closo*-carborane cage.

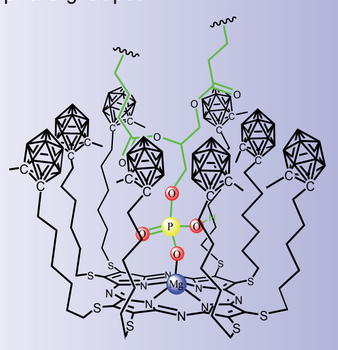


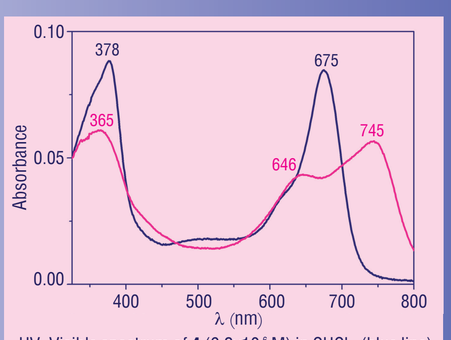
FT-IR spectrum of **4** (film on KBr)



UV–Visible spectrum of **5** ($6.2x10^{-6}$ M)

The electronic absorption spectrum of MgMCHESPz in $CHCl_3$ showed a clear split Q band and blue shifted B band, which are signatures of the remarkable tendency of the complex to self-aggregate in organic solvents. Aggregation could promptly be disrupted, however, upon addition of equimolar amounts of methanol, pyridine, or phospholipid, thus suggesting that the complex, when incorporated in the liposomal matrix, set up effective interactions with the polar heads of the lipids, particularly with the phosphate groups.

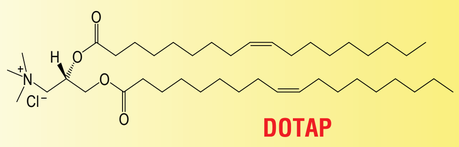

Proposed interaction mode between MgMHECSPz and the polar head of DOPA

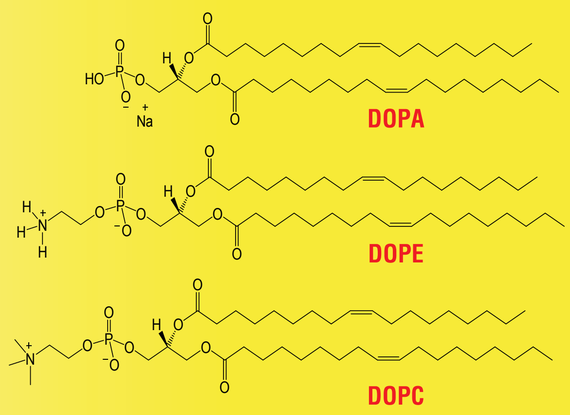


UV–Visible spectrum of **4** ($6.2x10^{-6}$ M) in $CHCl_3$ (blue line) and $CHCl_3$/MeOH (99:1 v/v) (pink line)

Pure and loaded liposomes

FORMULATION: Liposomes were made with different lipids , i.e. the positively charged DOTAP, the zwitterionic DOPC and the negatively charged DOPA, in order to obtain positive, zwiterionic and negative aggregates, respectively. In addition to this, all liposomes contained the zwitterionic phospholipid DOPE as a fusogenic element.


DOTAP



CHARACTERIZATION: SIZE AND CHARGE

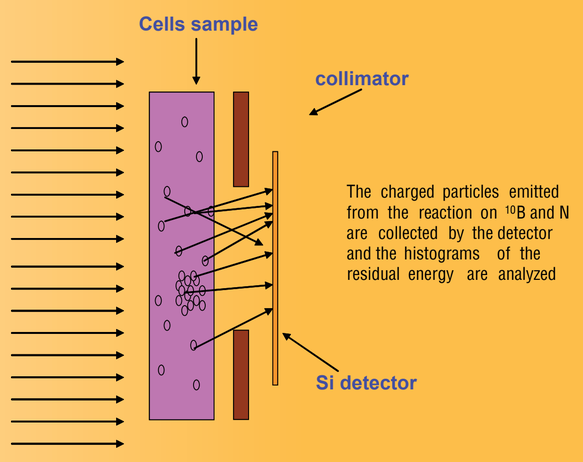
SAXS (Small Angle X-Ray Scattering) and SANS (Small Angle Neutron Scattering) experiments showed that progressive insertion of H_2MCHESPz affected the host liposomes, but did not disrupt their basic structure nor modified their monolamellar nature.
The mean size and size distribution width (polidispersity index) of plain and loaded liposomes were measured by Dynamic Light scattering (DLS).
The surface charge was evaluated through zeta potential measurements. This parameter is extremely important in the interaction between loaded liposomes

* **Pure liposomes** extruded with 100 nm pore diameter membranes have typical diameter 120 ± 10 nm and P.I. ~ 0.10 - 0.15. The Zeta potential depends on the lipid charge and has the following typical values:
DOTAP/DOPE ~ 30 - 40 mV
DOPC/DOPE ~ -20 - -30 mV
DOPA/DOPE ~ -60 - -70 mV

* **H_2MCHESPz loaded liposomes** undergo marked increase in the overall liposome size (mean diameter ~ 150-180 nm). The zeta potential tends to be reduced in absolute value, which is in agreement with the insertion of a neutral molecule and with the increased surface.

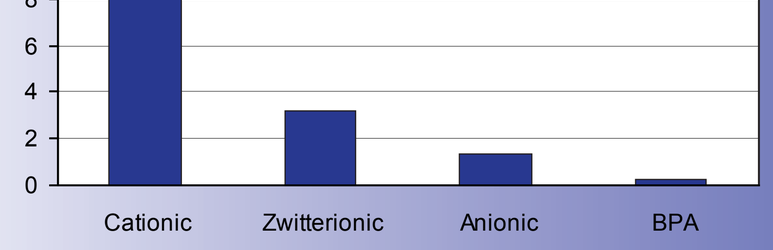
Boron uptake evaluation

Cells were treated with the ^{10}B enriched compounds and after 4 h incubation were washed three times in PBS, trypsinized, and resuspended in boron free medium. Enrichment by centrifugation was subsequently performed, and finally the cell pellet was layered on mylar discs. The intracellular boron concentration was detrmined by α-spectrometry. Irradiation was carried out with thermal neutrons at the TRIGA MARK II reactor (flux ~ 2 x 10^9 cm^{-2} s^{-1}) located at the University of Pavia (Italy).



In vitro studies

Cationic liposomes show the best carrier capability and allow to obtain ^{10}B cell concentration in the range of five-tenfold with respect to the starting delivery systems. This represents an improvement of at least 30 times in comparison with BPA (borophenylalanine), i.e. the approved drug for clinical trials.



[1]Ratio uptake of liposomes loaded with H_2MCHESPz for DHD cell lines

Conclusions and perspectives

New porphyrazines bearing peripheral *o*-carborane cages have been synthesized from readily available starting materials. Carboranyl-hexylthio-porphyrazines (CHESPzs) have proven to be efficiently incorporated in liposomes with tunable lipidic formulations, and hence, they do meet an essential prerequisite for being effectively delivered through the membrane of cancerous tissues. Preliminary studies on carboranyl-porphyrazines indicate that these compounds show negligible cell toxicity and, compared with BPA, a good cellular uptake, which encourages further studies for their evaluation as potential BNCT sensitizers.

References

1. (a) Ristori, S.; Salvati, A.; Martini, G.; Spalla, O.; Pietrangeli, D.; Rosa, A.; Ricciardi, G. *J. Am. Chem. Soc.* **2007**; *129*, 2728-2729; (b) Salvati, A; Ristori, S.; Martini, G.; Oberdisse, J.; Spalla, O.; Ricciardi, G.; Pietrangeli, D. ; Giustini, M.; Martini, G. *J. Phys. Chem. B* **2007**, *111*, 10357-10364; (c) Salvati, A; Ristori, S.; Pietrangeli, D.; Oberdisse, J.; Calamai, L.; Martini, G.; Ricciardi, G. *Biophys. Chem.* **2007**, *131*, 43-51.
2. Pietrangeli, D.; Ricciardi, G. *Appl. Rad. Isotopes* **2009**, *67*, 97-100.
3. Altieri, S.; Balzi, M.; Bortolussi, S.; Bruschi, P.; Ciani, L.; Clerici, A. M.; Faraoni, P.; Ferrari, C.; Gadan, M. A.; Panza, L.; Pietrangeli, D.; Ricciardi, G.; Ristori, S. *J. Med. Chem.* ***2009***, *52*, 7829-7835.