Synthesis of Novel Azapyridinomacrocyclic Trifunctional Chelatants for Biomolecules Labelling

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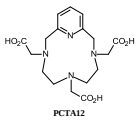
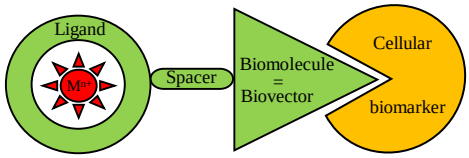
The coordination of metal ions is a method frequently used to label biomolecules as *biovectors* targeted to a specific cellular *biomarker*. Depending on the selected metal ion, the resulting targeted drug will find applications in medicine as diagnostic or therapeutic agents. For such purposes, multifunctional *ligands* are required in order to insure a strong and fast complexation of the metal ion on the one hand, and to enable a covalent grafting to the biomolecule on the other hand.

Polyamino-polycarboxylic compounds are known to give stable complexes avoiding any transchelation with endogenous cations as well as transmetalation with seric proteins, so that they are widely used in the medical area.[1]

In order to prepare potential MRI contrast agents, we have previously synthesized such ligands based on the macrocyclic PCTA12-scaffold to immobilize Gd^{3+}.[2-5] The research presented here describes preliminary results of the synthesis of novel PCTA12-based trifunctional prochelators for potential multi-modal medical applications.



Labelled biomolecules as targeted drugs



Metal ions

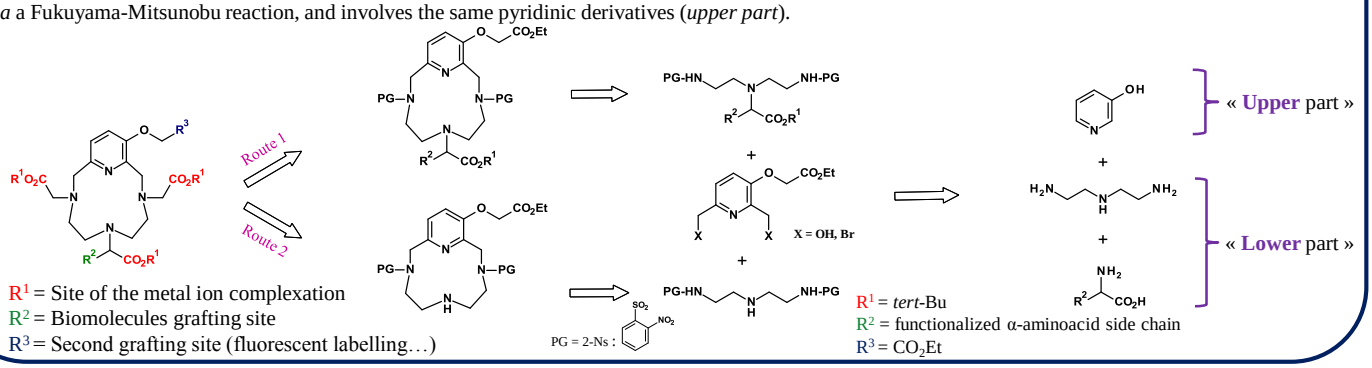
$^{111}In^{3+}$, $^{67}Ga^{3+}$, …
$(Al^{18}F)^{2+}$, $^{68}Ga^{3+}$, …
$^{90}Y^{3+}$, $^{153}Sm^{3+}$, $^{177}Lu^{3+}$, …

Medical Applications

SPECT imaging
PET imaging
Radiotherapy

PCTA12-based trifunctional prochelators - Retrosynthesis

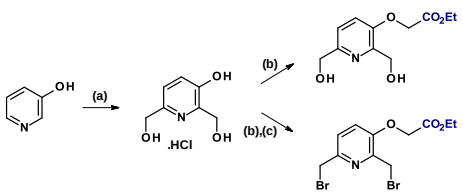
Routes 1 and 2 differ by the stage at which the part bearing the grafting site for the biomolecule and one of the three sites of the metal ion complexation is introduced. In route 1, it occurs later on a versatile macrocyclic synthon. In both routes, the macrocyclization step can be envisaged either *via* a S_N process or *via* a Fukuyama-Mitsunobu reaction, and involves the same pyridinic derivatives (*upper part*).



R^1 = Site of the metal ion complexation
R^2 = Biomolecules grafting site
R^3 = Second grafting site (fluorescent labelling…)

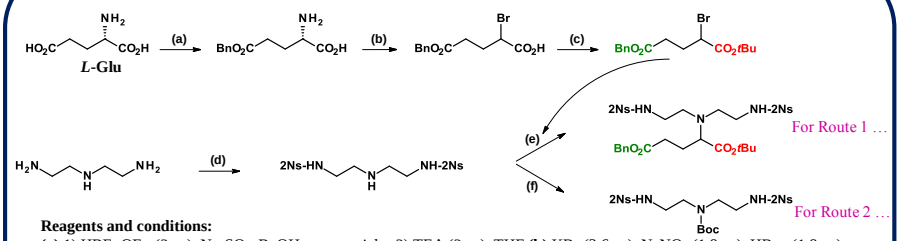
R^1 = *tert*-Bu
R^2 = functionalized α-aminoacid side chain
R^3 = CO_2Et

Synthesis of the « upper part »



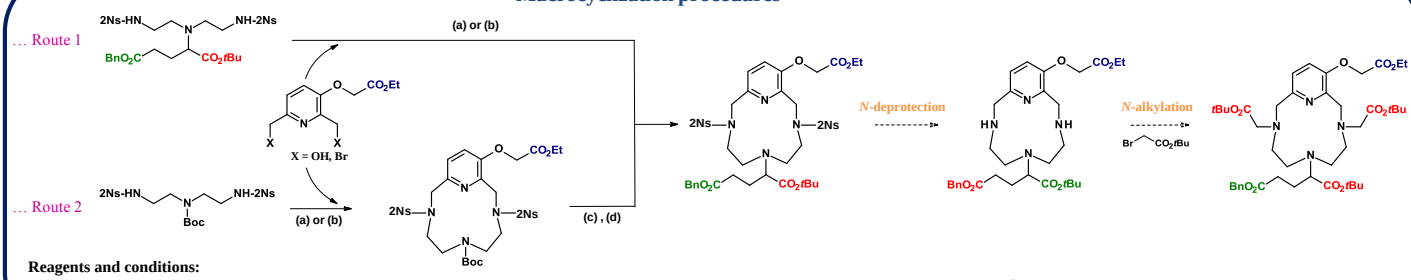
Reagents and conditions:
(a) 1) CH_2O (4.2 eq), NaOH (1 eq), H_2O, 90°C, 6h; 2) CH_3COOH (1.4 eq), HCl (1.5 eq); 81 %
(b) $BrCH_2COOEt$ (1.2 eq), K_2CO_3 (2.5 eq), CH_3CN, reflux, 6h; 33 %
(c) CBr_4 (2.2 eq), PPh_3 (2.2 eq), CH_3CN, rt, 24h; 55 %

Synthesis of the « lower part »



Reagents and conditions:
(a) 1) $HBF_4.OEt_2$ (2 eq), Na_2SO_4, BnOH, rt, overnight; 2) TEA (2 eq), THF **(b)** KBr (3.6 eq), $NaNO_2$ (1.8 eq), HBr_{aq} (1.8 eq), -10°C, 2h **(c)** $HClO_4$ (0.04 eq), *Act*Bu, rt, 7h; 18-21 % (3 steps)
(d) *o*-$NO_2(C_6H_4)SO_2Cl$ (2 eq), NaOH (2 eq), Et_2O/THF, rt, overnight; 62-86 % **(e)** $C_{16}H_{21}BrO_4$ (1.4 eq), K_2CO_3 (3.5 eq), THF, reflux, overnight; 36 % **(f)** Boc_2O (1.2 eq), THF, rt, 24h; 93 %

Macrocyclization procedures

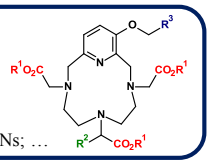


Reagents and conditions:
(a) For X = OH (1 eq): PBu_3 (3 eq), DIAD (3 eq), THF_{anh}, rt, 24 h; 45 % (Route 1); 65 % (Route 2); **(b)** For X = Br (1.2 eq): K_2CO_3 (4 eq), CH_3CN, reflux, 9h; 61 % (Route 1); 76 % (Route 2)
(c) TFA (27 eq), CH_2Cl_2, rt, overnight; 97 % **(d)** $C_{16}H_{21}BrO_4$ (3 eq), K_2CO_3 (4 eq), KI (0.25 eq), CH_3CN_{anh}, reflux, 48h; 44 %

Perspectives

- Last two synthetic steps required (*N*-deprotection/ *N*-alkylation) to obtain a first prochelator (R^1= *tert*-Bu, R^2= $(CH_2)_2CO_2Bn$, R^3=CO_2Et)
- Orthogonal protective groups: cleavage tests
- Measurement of the stereochemistry of the prochelators prepared (chiral derivatization)
- Functional modulation of grafting sites R^2, and R^3

R^2 =$(CH_2)_4$-NHCbz; …
R^3= COX-$(CH_2)_2$-NH-2Ns with X= O,N; CH_2-NH-2Ns; …



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2) F. Dioury, C. Ferroud, A. Guy, M. Port, *Tetrahedron* **2009**, *65*, 7573-7579.
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