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Cyclodextrin- and calixarene-based polycationic amphiphiles as gene delivery systems: a structure-activity relationship study

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Original
Cyclodextrin- and calixarene-based polycationic amphiphiles as gene delivery systems: a
structure-activity relationship study / Gallego Yerga, Laura; Lomazzi, Michela; Franceschi, Valentina;
Sansone, Francesco; Ortiz Mellet, Carmen; Donofrio, Gaetano; Casnati, Alessandro; García Fernández, José M.. - In: ORGANIC \& BIOMOLECULAR CHEMISTRY. - ISSN 1477-0520. - 13:6(2015), pp. 1708-1723.
[10.1039/C4OB02204A]
Availability:
This version is available at: 11381/2787200 since: 2015-04-02T09:58:44Z
Publisher:
Royal Society of Chemistry
Published
DOI:10.1039/C4OB02204A

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## Supporting Information

Cyclodextrin- and Calixarene-based Polycationic Amphiphiles as Gene Delivery Systems: A Structure-Activity Relationship Study

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## General methods

Preparation of pDNA complexes with synthetic vectors. The preparation of the DNA complexes from the CD or $\mathrm{CA}_{4}$ and reference vectors bPEI (branched polyethyleneimine) and LTX (Lipofectamine) has been performed according to a procedure which has been detailed elsewhere. ${ }^{1}$ Luciferase-enconding pDNA pTG11236 (pCMV-SV40-luciferaseSV40pA, 5739 base pairs) and Green Fluorescent Protein-enconding pDNA (pEGFP-C1, 4731 base pairs). The quantities of compound used were calculated according to the desired DNA concentration of $0.02 \mathrm{mg} \cdot \mathrm{mL}^{-1}$ or $0.07 \mathrm{mg}^{2} \mathrm{~mL}^{-1}$ (i.e. $60 \mu \mathrm{M}$ or $200 \mu \mathrm{M}$ phosphate, respectively), the $\mathrm{N} / \mathrm{P}$ ratio ( $1,2,5,10,20$ or 50 ), the molar weight, and the number of protonable nitrogens in the selected CD or calix[4]arene derivative or cationic polymer (bPEI) and the concentration of the vector was in the range 1.25-25 $\mu$ M.. First, pDNA was diluted in HEPES ( $20 \mathrm{mM}, \mathrm{pH} 7.4$ ) to a final concentration of 60 or $200 \mu \mathrm{M} \mathrm{PO}_{4}{ }^{3-}$. Then the desired amount of CD or $\mathrm{CA}_{4}$ derivative was added from 3,10 or 20 mM stock solution (DMSO) and bPEI and LTX was added from a 0.1 M stock solution $\left(\mathrm{H}_{2} \mathrm{O}\right)$. The preparation was vortexed, gently stirred for 2 h and used for characterization or transfection experiments.

Agarose gel electrophoresis. Each vector:pDNA complex ( $20 \mu \mathrm{~L}, 0.4 \mu \mathrm{~g}$ of plasmid) was submitted to electrophoresis for about 30 min under 150 V through a $0.8 \%$ agarose gel in TAE 1X (Tris-acetate-EDTA) buffer and stained by spreading GelRed Nucleic Acid Stain (Biotium). The DNA was then visualized after photographing on an Alphaimager Mini UV transilluminator. The plasmid integrity in each sample was confirmed by electrophoresis after decomplexation with sodium dodecyl sulfate (SDS, 8\%).

Hydrodynamic diameter and $\zeta$-potential of the CDplexes. The average sizes of the CDplexes were measured using a Zetasizer Nano ZS (Malvern Instruments) with the following specification: sampling time, automatic; number of measurements, 3 per sample; medium viscosity, 1.054 cP ; refractive index, 1.33 ; scattering angle, $173^{\circ} ; \lambda=633 \mathrm{~nm}$; temperature, $25^{\circ} \mathrm{C}$. Data were analyzed using the multimodal number distribution software included in the instrument. Results are given as volume distribution of the major population by the mean diameter with its standard deviation. No significant differences were encountered when the data were expressed in intensity or number distributions. This is
consistent with a spherical topology of the nanoparticles. $\zeta$-Potentials were determined using the "mixed-mode measurement" phase analysis light scattering (M3-PALS) following the specifications used for hydrodynamic diameter measurements. Before each series of experiments, the performance of the instruments was checked with either a 90 nm monodisperse latex beads (Coulter) for DLS or with DTS 50 standard solution (Malvern) for $\zeta$-potentials.

Transmission Electron Microscopy (TEM). Formvar-carbon coated grids ( $\mathrm{Cu}, 200$ mesh), previously made hydrophilic by glow discharge, were negatively stained with $1 \%$ aqueous solution of uranyl acetate. After 5 min of contact with a drop of that solution, the drop were removed and the grids were dried at rt for 24 h . Then, the grids were placed on top of small drops of the samples of CDplexes or CAplexes (HEPES $20 \mathrm{mM}, \mathrm{pH} 7.4$, DNA $303 \mu \mathrm{M}$ phosphate) prepared as described above. After 5 min of contact, the drops were removed and the grids were dried for 24 h . They were then observed with a Philips CM200 electron microscope working under standard conditions. All these experiments were reproduced twice on each formulation.

Atomic Force Microscopy (AMF) for complexes with DNA. Sample preparation and AFM imaging. DNA samples were prepared by diluting the plasmid DNA (pEGFP-C1, 4731 base pairs) to a final concentration of 0.25 nM in deposition buffer ( 20 mM HEPES, $10 \mathrm{mM} \mathrm{NaCl}, 2 \mathrm{mM}, \mathrm{MgCl}_{2}, \mathrm{pH}=7.4$ ) either in the presence or absence of compounds. To formulate the complexes, the desire amount of calixarene or cyclodextrine derivative was added from $10-50 \mathrm{mM}$ stock solutions ( DMSO or $\mathrm{H}_{2} \mathrm{O}$ ), to obtain samples at $\mathrm{N} / \mathrm{P}$ ratio from 0.5 to 10 and concentrations of compound from $0.08 \mu \mathrm{M}$ to $170 \mu \mathrm{M}$. The mixture was incubated for 5 min at room temperature. Then a $20 \mu \mathrm{~L}$ droplet was deposited onto freshlycleaved ruby mica (Ted Pella, Redding, CA) for 1.5 min . The mica disk was rinsed with milliQ water and dried with a weak nitrogen stream. AFM imaging was performed on the dried sample with a Nanoscope IIIA Microscope (Digital Instruments Inc. Santa Barbara, $C A$ ) operating in tapping mode. Commercial diving board silicon cantilevers (NSC-15 Micromash Corp., Estonia) were used. Images of $512 \times 512$ pixels were collected with a scan size of $2 \mu \mathrm{~m}$ at a scan rate of 3-4 lines per second and were flattened after recording using Nanoscope software.

Transfection experiments in COS-7 cell line using luciferase-encoding pDNA pTG11236. COS-7 cells (African green monkey kidney cells; ATCC, \#CRL-1651) were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10\% (v/v) heat-inactivated fetal bovine serum (FBS, Sigma) and $1 \%$ ( $\mathrm{v} / \mathrm{v}$ ) penicillinstreptomycin (Sigma), at $37^{\circ} \mathrm{C}$ under a $5 \% \mathrm{CO}_{2}$ atmosphere. The cells were harvested by tripsinization three times a week using Trypsin/EDTA (Sigma) and phosphate-buffered saline (PBS, Dulbeccos's Phosphate B., Sigma) was use to wash cells.

Twenty-four hours before transfection, COS-7 cells were grown at a density of $2 \cdot 10^{4}$ cells/well in a 96 -well plates in the suitable complete culture media, in a wet $\left(37^{\circ} \mathrm{C}\right)$ and $5 \% \mathrm{CO}_{2} / 95 \%$ air atmosphere. The above CD or $\mathrm{CA}_{4}: p D N A$ complexes and PEI:pDNA polyplexes, previously formulated and incubated for 2 h as described above, were diluted to $100 \mu \mathrm{~L}$ in DMEM or in DMEM supplemented with $0 \%-10 \%$ fetal bovine serum in order to have $0.5 \mu \mathrm{~g}$ of pDNA in the well ( $15 \mu \mathrm{M}$ phosphate).

The culture medium was removed and replaced by these $100 \mu \mathrm{~L}$ of complexes and it was incubated at $37^{\circ} \mathrm{C}$ under at $5 \% \mathrm{CO}_{2}$ atmosphere. After 4 h and $24 \mathrm{~h}, 50$ and $100 \mu \mathrm{~L}$ of DMEM supplemented with $10 \%$ FBS were added. After 48 h , the transfection was stopped, the culture medium was discarded, and the cells washed twice with $100 \mu \mathrm{~L}$ of PBS and lysed with $50 \mu \mathrm{~L}$ of lysis buffer (Promega, USA). The lysates were frozen at $-80^{\circ} \mathrm{C}$ before the analysis of luciferase activity. This measurement was performed in a luminometer (Synergy HT, BioTek) for 10 s on $20 \mu \mathrm{~L}$ on the lysis mixture and using the luciferase determination system (Promega) in 96-well plates. The total protein concentration per well was determined using a protein assay kit (BioRad). Luciferase activity was calculated as nanograms (ng) of luciferase per mg of protein (based on a standard curve for luciferase activity). The data were calculated from three or four repetitions in two fully independent experiments (formulation and transfection).

Transfection experiments in COS-7 and RD-4 cell lines using green fluorescent protein-encoding pDNA pEGFP-C1. RD-4, human Rhabdomyosarcoma cell line (obtained from David Derse, National Cancer Institute, Frederick, MD) and COS-7 cell line (African green monkey kidney cells; ATCC, \#CRL-1651) were grown in EMEM medium containing NEAA, $10 \%$ fetal bovine serum, $2 \mathrm{mM} \mathrm{L-glutamine} ,100 \mathrm{IU} \mathrm{mL}^{-1}$ penicillin and
$100 \mu \mathrm{~g} \mathrm{~mL}$ - streptomycin. All cultures were incubated at $37^{\circ} \mathrm{C}$ in a humidified atmosphere containing $5 \% \mathrm{CO}_{2}$. Cells were subcultured to a fresh culture vessel when growth reached $70-90 \%$ confluence (that is, every $3-5$ days) and incubated at $37^{\circ} \mathrm{C}$ in a humidified atmosphere of $95 \%$ air and $5 \% \mathrm{CO}_{2}$. Transfections were performed in 24 -well plates, when cells were $80 \%$ confluent ( $\sim 5 \times 10^{4}$ cells) on the day of transfection. Plasmid $(2.5 \mu \mathrm{~g}$ corresponding to 1 nM ) and different concentrations of ligands were added to 1 mL of serum-free medium (DMEM, 2 mM L-glutamine and $50 \mu \mathrm{~g} \mathrm{ml}{ }^{-1}$ ), mixed rapidly and incubated at room temperature for 20 min . Following the removal of the culture medium from the cells, 0.5 mL of transfection mixture were carefully added to every well. Lipoplex formulations with helper lipid were prepared adding a 2 mM ethanol solution of DOPE to plasmid-vector mixture at $1: 2$ vector-DOPE molar ratio. Vector concentration was in the range $1.25-10 \mu \mathrm{M}$. These solutions administered to the cells were completely clear and homogeneus. LTX and PEI transfection reagent were used according to manufacturer's protocol as a positive transfection control.

The mixture and cells were incubated at $37^{\circ} \mathrm{C}$ in a humidified atmosphere of $95 \%$ air and $5 \% \mathrm{CO}_{2}$ for 5 h . Finally, transfection mixture was removed and 1 mL of growth medium added to each transfected well and left to incubate for 72 h . Five fields were randomly selected from each well without viewing the cells (one in the centre and one for each quadrant of the well) and examined. The transfected cells were observed under fluorescence microscope for enhanced green fluorescent protein expression. Each experiment was done at least three times.

In all transfection experiments, statistical differences between treatments were calculated with student's $t$-test and multifactorial analysis of variance.

Total protein method. BioRad $D C^{T M}$ total protein assay was used. The percentage of cell viability was calculated as the ratio of the total protein amount per well of the transfected cells relative to that measured for untreated cells x 100. The data were calculated from three or four repetitions in two fully independent experiments (formulation and transfection).

MTT assay. For MTT assay $3 \cdot 10^{3}$ cells (RD4 or COS-7) were seeded in 96 well plates and incubated at $37^{\circ} \mathrm{C}$, in a humidified atmosphere containing $5 \% \mathrm{CO}_{2}$ till confluence. $50 \mu \mathrm{~L}$ per well of the selected dilution of the $\mathrm{CA}_{4}$ or CD derivatives were then added, usually
compounds were tested in a range from 40 to $2.5 \mu \mathrm{M}$ with or without DOPE (keeping the molar ratio $1: 2$ vector-DOPE). The plates were incubated for 24 hours at $37^{\circ} \mathrm{C}, 5 \% \mathrm{CO}_{2}$. $10 \mu \mathrm{~L}$ per well of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were then added at the final concentration of $5 \mathrm{mg} / \mathrm{mL}$ (MTT can be diluted in sterile PBS or in medium) and after an incubation of 6 hours $100 \mu \mathrm{~L}$ per well of a lysant ( $10 \%$ SDS and 0.01 M HCl in $\mathrm{H}_{2} \mathrm{O}$ ) were added. Plates were sealed and incubated overnight at $37^{\circ} \mathrm{C}, 5 \%$ $\mathrm{CO}_{2}$. The absorbance was read at 620 or 540 nm after shaking of the plates. The rate of toxicity/survival was calculated respect to the controls, the cell line incubated in absence of the compound.

Statistical Analysis. Statistical analyses were performed using SPSS software from SPSS Inc. (Chicago, IL, USA). Statistical differences were calculated with student's $t$-test and multifactorial analysis of variance.

Table S1. Hydrodynamic sizes ( nm ) $\pm$ SD and polydispersity index of complexes pDNApolycationic amphiphilic derivatives.

| Compound | Size <br> $(\mathbf{n m}) \mathbf{N} / \mathbf{P} \mathbf{5}$ | PI | Size <br> $(\mathbf{n m}) \mathbf{N} / \mathbf{P ~ 1 0}$ | PI |
| :---: | :---: | :---: | :---: | :---: |
| $\mathbf{1 a}$ | $108.6 \pm 2.2$ | $0.22 \pm 0.02$ | $85.89 \pm 2.2$ | $0.20 \pm 0.01$ |
| 1b | $109.3 \pm 1.6$ | $0.20 \pm 0.01$ | $81.2 \pm 0.7$ | $0.21 \pm 0.01$ |
| 1c | $113.9 \pm 1.0$ | $0.21 \pm 0.02$ | $97.8 \pm 2.1$ | $0.21 \pm 0.01$ |
| 2a | $117.7 \pm 1.3$ | $0.30 \pm 0.01$ | $93.07 \pm 1.2$ | $0.24 \pm 0.01$ |
| 2b | $123.1 \pm 3.3$ | $0.40 \pm 0.02$ | $87.35 \pm 0.7$ | $0.32 \pm 0.01$ |
| 2c | $121.6 \pm 3.0$ | $0.27 \pm 0.01$ | $92.74 \pm 0.4$ | $0.16 \pm 0.03$ |
| 1d | $109.3 \pm 0.8$ | $0.26 \pm 0.02$ | $82.6 \pm 3.9$ | $0.27 \pm 0.04$ |
| 1e | $112.4 \pm 1.1$ | $0.25 \pm 0.01$ | $83.8 \pm 3.6$ | $0.37 \pm 0.03$ |
| 2d | $87.6 \pm 0.3$ | $0.23 \pm 0.01$ | $81.2 \pm 0.4$ | $0.23 \pm 0.02$ |
| 2e | $84.5 \pm 0.8$ | $0.24 \pm 0.01$ | $95.5 \pm 0.7$ | $0.34 \pm 0.01$ |
| 1f | $157.8 \pm 3.4$ | $0.23 \pm 0.02$ | $92 \pm 3.7$ | $0.34 \pm 0.01$ |
| 2f | $174.1 \pm 6.4$ | $0.42 \pm 0.06$ | $116.6 \pm 5.7$ | $0.33 \pm 0.05$ |

Table S2. $\zeta$ Potential $(\mathrm{mV}) \pm$ SD of complexes pDNA-polycationic amphiphilic derivatives.

| Compound | N/P $\mathbf{5}$ | N/P 10 |
| :---: | :---: | :---: |
| $\mathbf{1 a}$ | $59.2 \pm 1.2$ | $61 \pm 1.1$ |
| $\mathbf{1 b}$ | $58.5 \pm 2.2$ | $71.2 \pm 1.3$ |
| 1c | $55.6 \pm 2.6$ | $63.2 \pm 2.9$ |
| 2a | $43.1 \pm 1.1$ | $50.8 \pm 1.6$ |
| 2b | $46.8 \pm 2.2$ | $50 \pm 2.1$ |
| 2c | $42.6 \pm 0.7$ | $53.5 \pm 2.0$ |
| 1d | $55.8 \pm 0.3$ | $62.1 \pm 2.4$ |
| 1e | $50.9 \pm 1.9$ | $54.1 \pm 2.3$ |
| 2d | $46.5 \pm 1.1$ | $54.8 \pm 1.7$ |
| 2e | $45.7 \pm 1.6$ | $52.7 \pm 1.0$ |
| $\mathbf{1 f}$ | $40.1 \pm 1.1$ | $44.1 \pm 0.8$ |
| 2f | $29.5 \pm 1.2$ | $46.5 \pm 1.6$ |



Figure S1. Transfection experiments in COS-7 and RD-4 cells performed with pEGFP-C1 plasmid 1 nM and guanidinium derivatives (1f, 2f) with and without DOPE (1:2 molar ratio vector-DOPE), 10/ $20 \mu \mathrm{M}$ ). Transfected cells are visualized with fluorescence microscopy (right row, in light green because they express the enhanced green fluorescence protein EGFP) and phase contrast microscopy (lower row).


Figure S2. Transfection experiments in COS-7 and RD-4 cells performed with pEGFP-C1 plasmid 1 nM and guanidinium derivatives ( $\mathbf{1 f}, \mathbf{2 f}$ ) with and without DOPE (1:2 molar ratio vector-DOPE), $10 / 20 \mu \mathrm{M}$ ). Transfected cells are visualized with fluorescence microscopy (right row, in light green because they express the enhanced green fluorescence protein EGFP) and phase contrast microscopy (lower row).


Figure S3. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $400 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) of compound 4.


Figure S4. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $400 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 5 .


Figure S5. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $500 \mathrm{MHz}, 125.7 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 2a.




Figure S6. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 75.5 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 6 .


Figure S7. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 125.7 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound $\mathbf{2 b}$.


Figure S8. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $400 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 7.


Figure S9. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 75.5 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 2c.


Figure S10. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $500 \mathrm{MHz}, 125.7 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound $\mathbf{1 0}$.


Figure S11. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $500 \mathrm{MHz}, 125.7 \mathrm{MHz}$, DMSO- $\mathrm{d}_{6}$ ) of compound 1d.


Figure S12. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) of compound 11.





Figure S13. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $500 \mathrm{MHz}, 125.7 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 13 .


Figure S14. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $500 \mathrm{MHz}, 125.7 \mathrm{MHz}$, DMSO- $\mathrm{d}_{6}$ ) of compound $\mathbf{1 e}$.


Figure S15. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) of compound 15 .


Figure S16. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 16.


Figure S17. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $400 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) of compound 17 .


Figure S18. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 75 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) of compound 18.


Figure S19. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $500 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 19.


Figure S20. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $500 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound $\mathbf{2 e}$.


Figure S21. ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectra ( $400 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) of compound 20.


Figure S22. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 75.5 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound 21.


Figure S23. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 125.7 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound $\mathbf{1 f}$.


Figure S24. ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectra ( $300 \mathrm{MHz}, 75.5 \mathrm{MHz}, \mathrm{CDCl}_{3}$ ) of compound 22.


Figure S25. ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectra ( $400 \mathrm{MHz}, 100.6 \mathrm{MHz}, \mathrm{CD}_{3} \mathrm{OD}$ ) of compound $\mathbf{2 f}$.





Figure S26. ESI-MS spectra of compounds $2 \mathbf{2 a}$ (A), 2b (B), 2c (C), 2e (D), $\mathbf{1 f}$ (E) and $\mathbf{2 f}$ (F).


Figure S27. MALDI-TOF spectra of compounds 1d (A) and 2d (B).

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[^0]:    ${ }^{1}$ A. Díaz-Moscoso, L. Le Gourriérec, M. Gómez-García, J. M. Benito, P. Balbuena, F. OrtegaCaballero, N. Guilloteau, C. Di Giorgio, P. Vierling, J. Defaye, C. Ortiz Mellet, J. M. García Fernández, Chem. Eur. J. 2009, 15, 12871-12888.

