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Communication

Hyperbranched Molecular Structures with Potential Antiviral Activity: Derivatives of 5,6-Dihydroxyindole-2-Carboxylic Acid

Mario Sechi [1,*], Fabio Casu [1], Ilaria Campesi [1], Stefano Fiori [2] and Alberto Mariani [2]

[1] Dipartimento Farmaco Chimico Tossicologico, Università di Sassari, Via Muroni 23/A, 07100 Sassari, Italy; E-mail: mario.sechi@uniss.it

[2] Dipartimento di Chimica, Università di Sassari and local INSTM Research Unit, Via Vienna 2, 07100 Sassari, Italy; E-mail: mariani@uniss.it

* Author to whom correspondence should be addressed: E-mail: mario.sechi@uniss.it ; Tel. +39 079 228 753; Fax. +39 079 228 720

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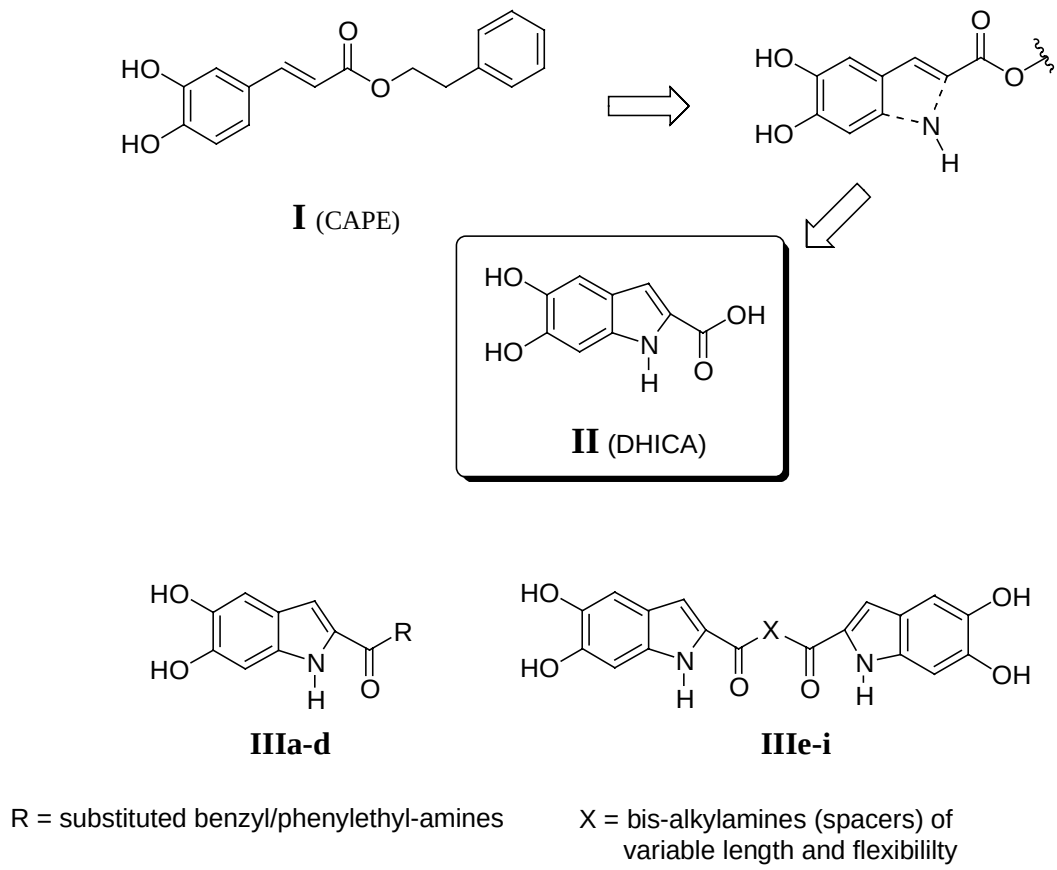
Abstract: In the search of new HIV-1 integrase (IN) inhibitors, we synthesized a series of multimeric 5,6-dihydroxyindole-2-carboxylic acid (DHICA) derivatives. Preliminary results indicate that hyperbranched architectures could represent a peculiar molecular requisite for the development of new antiviral lead compounds.

Keywords: Hyperbranched polymers; AB_2 monomers; DHICA; HIV-1; antiviral agents.

Introduction

In the search for new HIV-1 IN inhibitors, we synthesized and evaluated the biological activity of 5,6-dihydroxyindole-2-carboxylic acid (DHICA, **II**), an intermediate in the pathway of eumelanin production, and a series of its derivatives (**IIIa-i**, Figure 1). These compounds were designed as conformationally constrained analogues of the acrylate moiety of caffeic acid phenethyl ester (CAPE, **I**, Figure 1); several of them showed anti-IN activity in enzyme-based assays at low micromolar concentrations [1a,b].

Figure 1. Design of DHICA from CAPE and DHICA derivatives.



In the course of the preparation of **II** by a novel synthetic route (see below), we unexpectedly obtained a macromolecular compound as the only product. On the basis of its physico-chemical properties such as solubility and differential scanning calorimetry (DSC) measurements, its structure **IVa** has been postulated to perhaps correspond to that of a hyperbranched compound. In order to evaluate whether the macromolecular structure of **IVa** could be endowed with biological activity, we prepared other derivatives in both hyperbranched oligomeric (**IVb**) and polymeric (**IVc**) forms (Figure 2). Indeed, since **II** bears one COOH (A) and two OH (B) groups, which are capable of mutually reacting, it can be regarded as an AB_2 monomer, typically giving hyperbranched macromolecules (Figure 3).

Figure 2. Title compounds.

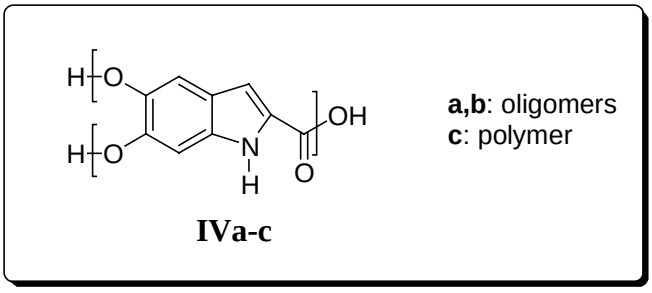
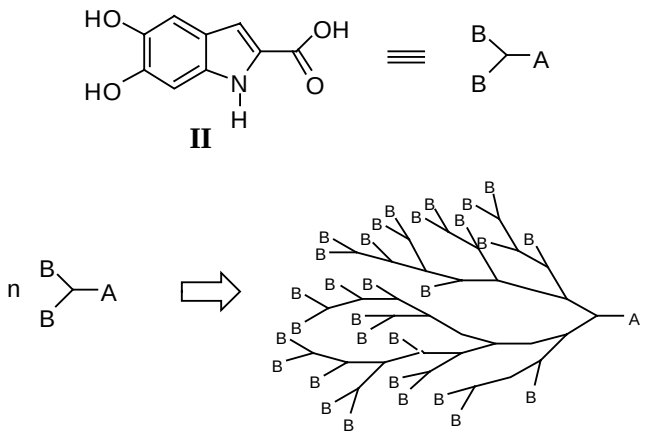


Figure 3. Schematic architecture of the hyperbranched macromolecules originating from AB_2 monomers.



In the last decades, AB_2 monomers have been widely used as starting materials for the synthesis of dendritic structures [2,3]. This macromolecular family comprises dendrimers and hyperbranched polymers. Both classes are characterized by having a tree-like architecture in which all linkages converge towards a central core. However, whereas dendrimers are obtained by stepwise growth in long sequences, hyperbranched polymers are obtained in one step. As far as dendrimers are concerned, their applications in treatment of several human diseases are under critical investigation and looks very promising; these macromolecules serve as targeted drug, carriers and delivery agents as well as imaging agents in human systems [4-6]. In particular, polymers having a dendrimer-based structure have emerged from several research programs focused on the development of inhibitors of HIV and other enveloped viruses [7-19].

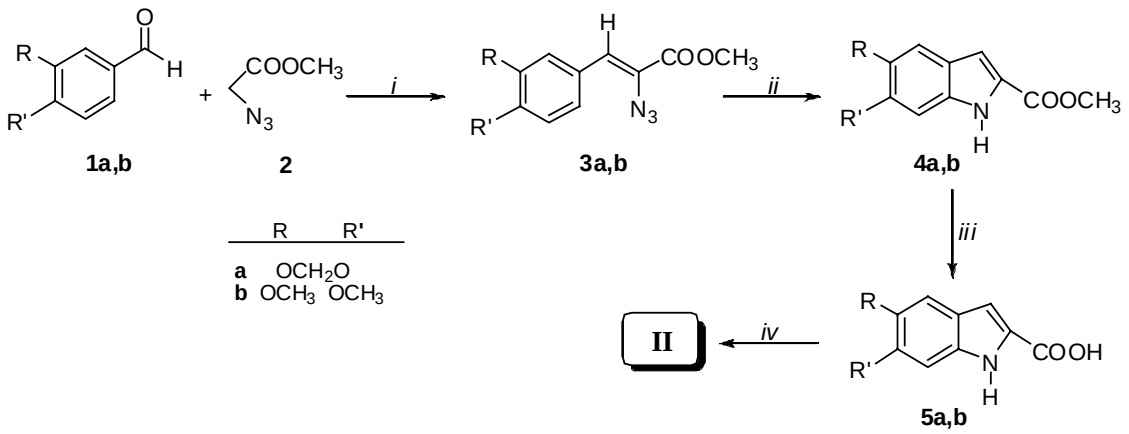
From a merely structural point of view, purification and deprotection steps for dendrimers are aimed at synthesizing monodisperse, defect-free macromolecules. On the contrary, hyperbranched polymers are characterized by a certain number of defects, which generally do not significantly affect their peculiar features. Although the synthetic chemistry efforts in modern drug research have been focused on the drive to discover orally bioavailable small-molecule drugs, the study of macromolecular entities could reveal novel and significant scenarios. For example, it is well-known that many of the biological targets are in fact macromolecules which rely heavily on polyvalent/multivalent interactions in their binding and signalling cascades. In this context, with the hope of identifying an original class of antiviral agents, all title compounds will be tested against HIV-1 viral strain.

Results and Discussion

Our synthetic approach to compounds **II** and **IVa-c** is depicted in Schemes 1-4. Starting from the aldehydes **1a** and **1b** and methyl azidoacetate (**2)**, azidocinnamates **3a** and **3b** were prepared in high yields via the Hemetsberger reaction [1a]. Intermediates **3a** and **3b** were converted in refluxing xylene into the esters **4a** and **4b**, from which the acids **5a** and **5b** were obtained in 98% yields on alkaline hydrolysis. Deprotection of the catechol moiety with BBr_3 in dichloromethane at –40 °C gave the expected DHICA **II** (Scheme 1). Compound **IVa** was obtained by demethylation of intermediate **4b** to

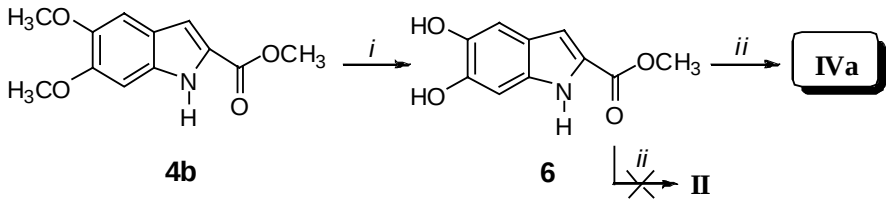
give methyl 5,6-dihydroxyindole-2-carboxylate (**6)**, which was treated with 12% KOH at reflux for 1 h (Scheme 2).

Scheme 1.



Reagents and conditions: i) CH_3ONa, CH_3OH, -15 °C for 4 h; ii) xylene, reflux for 15 min; iii) 12% KOH, reflux for 1 h; vi) BBr_3, CH_2Cl_2, H_2O, -40 °C for 4 h.

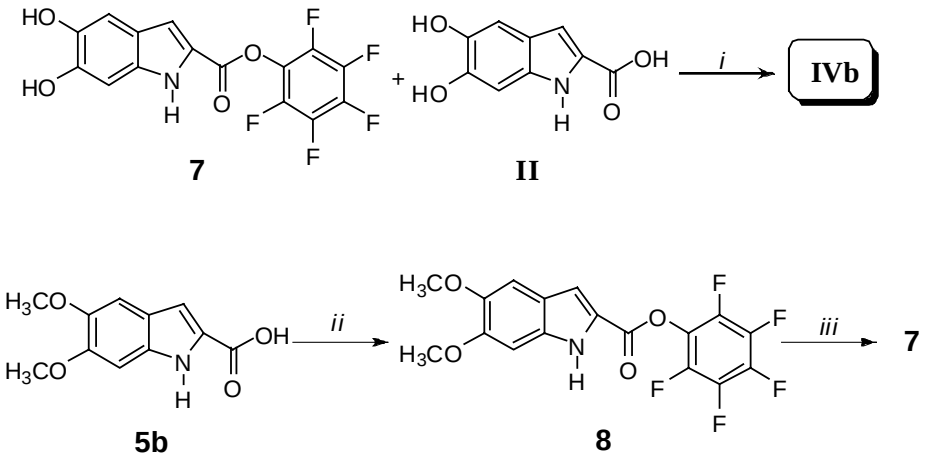
Scheme 2.



Reagents and conditions: i) BBr_3, CH_2Cl_2, CH_3OH, -40 °C, 4 h; ii) 12% KOH, reflux, 1 h.

It is noteworthy that, due to transesterification reactions, compound **II** was not obtained, thus confirming that oligomerization was favoured. The synthesis of **IVb** was carried out by reacting equimolar amounts of **7** and **II** in anhydrous DMF for 60 h, in the presence of a catalytic amount of triethanolamine (Scheme 3).

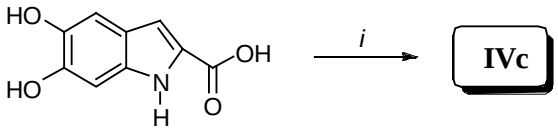
Scheme 3.



Reagents and conditions: i) Triethanolamine, anhydrous DMF, 80 °C, 60 h; ii) Pentafluorophenol, dioxane, r.t. for 4 h; iii) BBr_3, CH_2Cl_2, H_2O, -40 °C for 4 h.

The intermediate **7** was obtained by treating **5b** with pentafluorophenol to afford the pentaflurophenyl ester **8**. The latter was then deprotected to give **7** in good yield. Since hydroxy and pentafluorophenolic ester were the only reactive groups under such reaction conditions, a typical AB_2 + B_2 polymerization occurred, thus giving rise to dendritic oligomeric structures of relatively low molecular weight. This procedure was carried out with the aim of obtaining, by a different synthetic procedure, an oligomer comparable to **IVa**. As a matter of fact, when compared to **II**, both **IVa** and **IVb** showed a larger solubility in many polar solvents (water included). Besides, a further synthesis was designed and carried out: polymer **IVc** was prepared by reacting monomer **II** following a well-known polymerization route commonly used in the lab-scale *direct* preparation of high molecular weight polyesters from carboxylic acids (Scheme 4) [2].

Scheme 4.



II

Reagents and conditions: i) Anhydrous N-methyl pyrrolidone/pyridine (5:1, v/v); triphenyl-phosphine, hexachloroethane, 25 °C, 4 h.

Compound **IVc** was characterized by a solubility even larger than that of **II**, **IVa**, and **IVb**, thus suggesting the presence of more numerous OH groups and confirming a (highly-)branched structure which behaves as a unimolecular micelle. In order to obtain information about the polymeric structure of the title compounds, a full characterization of **IVa-c** is under way and will be reported elsewhere. However, some preliminary results can be here briefly outlined. DSC experiments show broad melting peaks for all samples, thus indicating the typical polydispersion state of a one-step-obtained synthetic oligo- or polymeric compound.

According to the expected increase of polydispersity as the molecular weight increases, typical for step-growth polymerization, melting peaks become larger in the following order: **IVa** < **IVb** << **IVc**; this behavior being due to the increasing number of different molecular species that characterize any of the above compounds. Furthermore, also melting temperatures give some indications; indeed, their values increase in the following order: **IVa** < **IVb** < **IVc** (i.e. 75 < 80 < 85 °C, respectively) that is what expected on the basis of the molecular weight of the three compounds, from the lowest to the highest.

Biological Activity

Preliminary biological data on cytotoxicity and antiviral activity of the title compounds are reported in Table 1. The compound concentrations required to reduce the viability of mock-infected cells by 50% (CC_{50}) and to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogenicity (EC_{50}), were determined by the MTT method [20]. In cell-based assays, **IVa-c** showed cytotoxicity and antiviral activity which were inversely related to their molecular weight. In particular, with comparable EC_{50} values, the oligomers **IVa** and **IVb** (EC_{50}s = 0.5 and 1.5 μg/mL for

IVa and **IVb**, respectively) seem significantly capable to prevent the HIV-1 multiplication in acutely infected MT-4 cells with respect to the polymer **IVc** (EC_{50} = >30 μg/mL). Meanwhile, cytotoxicity values support this observation (CC_{50}s = 5, 15, and >100 μg/mL for **IVa**, **IVb** and **IVc**, respectively). Interestingly, with respect to the monomer **II** and its derivatives **IIIa-i**, which were toxic and did not yield therapeutic efficacy [1a], the multimeric compounds have been proved to be effective in the HIV-1 multiplication, thus showing cytoprotection activity in cell-based assays. Although **IVa** and **IVb** proved significantly less potent than the reference compound Efavirenz (EC_{50} = 0.5 and 1.5 μg/mL *v*. 0.0031 μg/mL), a non-nucleoside reverse transcriptase inhibitor (NNRTI) commonly used in therapeutic protocols [21], they showed a similar biological profile when compared to Merck L-731,988 [22] (an important HIV-1 integrase inhibitor lead compound).

Table 1. Cytotoxicities and Antiviral Activities of **IVa-c**.

Cpd	[a]CC_{50}	[b]EC_{50}	[c]TI
II	0.9	>0.9	-
IVa	5	0.5	10
IVb	15	1.5	10
IVc	>100	>100	-
[d]**L-731,988**	15.6	0.43	36
[e]**EFV**	11	0.0031	>1000

[a]CC_{50}: Cytotoxic concentration 50%; [b]EC50: Effective Concentration 50%; [c]TI: Therapeutic index = CC_{50}/EC_{50}; [d]L-731,988: Merck's lead compound; [e]EFV: Efavirenz. Due to their oligo/polymeric nature, data referring to biological activities of multimeric derivatives (and those of the reference compounds for comparison) are expressed in μg/mL.

Conclusions

These preliminary results for **IVa,b** are consistent with the conclusion that a hyperbranched oligomeric backbone with relatively low molecular weight and relatively high degree of branching could represent an interesting structural archetype for HIV-1 inhibition as well as for other potential antiviral activities. Moreover, the possibility that hyperbranched derivatives might target the adsorption/fusion steps of HIV multiplication cycle in cells could be considered. Detailed cytotoxicity and antiviral activity as well as inhibition activities toward cellular targets of these compounds are under investigation and will be discussed in due course.

Experimental

General

The synthesis of monomer **II** and intermediates **3a**,**b**, **4a,b**, **5a,b**, **7** and **8** used for the preparation of the title compounds was previously reported [1a]. These compounds have been prepared by using

the same experimental procedure. Anhydrous solvents and all reagents were purchased from Aldrich, Merck or Carlo Erba. All reactions involving air- or moisture-sensitive compounds were performed under nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Melting points (m.p.) were determined using an Electrothermal melting point or a Köfler apparatus and are uncorrected. Infrared (IR) spectra were recorded as thin films or nujol mulls on NaCl plates with a Perkin-Elmer 781 IR spectrophotometer and are expressed in ν (cm^{-1}). Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were determined in 1:3 $CDCl_3$/DMSO-d_6 or DMSO-d_6 and were recorded on a 200 MHz Varian XL-200 instrument. Chemical shifts (δ scale) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) used as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; brs, broad singlet; dd, double doublet. The assignment of changeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was done on Merck silica gel F-254 plates. For flash chromatography Merck Silica gel 60 was used with a particle size 0.040-0.063 mm (230-400 mesh ASTM). Elemental analyses were performed on a Perkin-Elmer 2400 spectrometer, and were within ±0.4% of the theoretical values. Differential Scanning Calorimetry (DSC) measurements were performed using a DSC Q100 V9.0 (TA Instrument, New Castle, USA) as detailed below.

Preparation of methyl 5,6-dihydroxy-1H-indole-2-carboxylate (**6**). To a solution of 5,6-dimethoxy-1*H*-indole-2-carboxylic acid methyl ester **4b** (1.0 mmol) in dichloromethane (130 mL), a 1M BBr_3 sol. in dichloromethane (4 mmol) was added at -40 °C and under nitrogen atmosphere. The mixture was stirred at the same temperature for 4 h, then, the reaction was quenched in methanol and the solvents were removed under reduced pressure. The residue was washed three times with methanol. After purification by silica gel flash column chromatography, the product was recrystallized from isopropyl alcohol. Yield = 80 %; m.p. = 248 - 249 °C (lit. [23] 255 – 260 °C); IR (nujol) ν cm^{-1} = 3480 (OH); 3320 (NH); 1690 (C=O); ^{1}H-NMR ($CDCl_3$/DMSO-d_6) δ 10.98 (s, 1H, NH), 8.76 (s, 1H, OH), 8.35 (s, 1H, OH), 6.93 (s, 1H, Ar-H), 6.88 (s, 2H, Ar-H), 3.83 (s, 3H, CH_3); ^{1}H-NMR (DMSO-d_6) δ 11.28 (s, 1H, NH), 9.15 (s, 1H, OH), 8.65 (s, 1H, OH), 6.89 (s, 2H, Ar-H), 6.79 (s, 1H, Ar-H), 3.81 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6): δ 161.7, 146.5, 142.2, 132.9, 124.5, 119.8, 107.5, 104.9, 96.9, 51.3; Anal. Calcd. for $C_{10}H_9NO_4$: C, 57.97; H, 4.38; N, 6.76. Found: C, 58.11; H, 4.22; N, 6.89.

Preparation of **IVa**. In a 25 mL round bottom vessel, **6** (0.200 g, 0.960 mmol) was dissolved in 12% KOH (9 mL, 19.20 mmol) and the mixture was allowed to stir for 30 min at reflux. The dark solution formed was then poured onto ice and acidified with 6N HCl. Thereafter, the mixture was poured into diethyl ether and the precipitated oligomer **IVa** was filtered to provide a dark powder that was washed with water and diethyl ether and dried in a vacuum oven for 24 h at 40 °C. DSC: Tm = ~75 °C.

Preparation of **IVb**. Compound **II** (0.054 g, 0.27 mmol) and **7 (**0.096 g, 0.27 mmol) in anhydrous DMF (7.5 mL) containing anhydrous triethylamine (0.1 mL) were placed in a 10 mL round bottom vessel at room temperature. The vessel was sealed and heated in an oil bath set at 80 °C. The mixture was allowed to react under stirring for 60 h. The mixture was then poured into diethyl ether and the

precipitated oligomer **IVb** was filtered to give a dark powder that was washed with diethyl ether and dried in a vacuum oven for 24 h at 40 °C. DSC: Tm = ~80 °C.

Preparation of **IVc**. In a 5 mL round bottom vessel compound **II** (0.050 g, 0.26 mmol) was dissolved in an N-methylpyrrolidone/pyridine mixture (1.5 mL, 5:1, v/v) and triphenylphosphine (0.080 g, 0.49 mmol) and hexachloroethane (0.093 g, 0.57 mmol) were added. The vessel was sealed and the mixture was allowed to stir for 4 h at room temperature, then diethyl ether (15 mL) was added and the precipitated polymer **IVc** was recovered and washed several times by centrifugation in the presence of methanol to give a brown powder. DSC: Tm = ~85 °C.

Differential Scanning Calorimetry (DSC) measurements

The DSC instrument was calibrated with indium (calibration standard, purity 99.999%) for melting point and heat of fusion. A heating rate of 10 °C/min was employed in the range 20–250 °C. Analyses were performed under an Ar purge (50 mL/min). Standard aluminium sample pans were used. About 8-9 mg sample was taken for analysis. An empty pan was used as reference.

Acknowledgements

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Sample Availability: Samples of the compounds **I-IV** and **1-8** are available from authors.