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New Developments in the Synthesis of EMICORON

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Abstract

In this paper, the modification of two synthetic steps to obtain EMICORON, with respect to the usual protocol, is reported. EMICORON is a benzo[*ghi*]perylen-diimmide which was synthesized the first time in our laboratory in 2012 and showed to have in-vivo antitumor activities interfering with tumor growth and development by a multi-target mechanism of action. The provided modifications regarded the reaction times and the reaction conditions as well as the work-up procedures and allowed to increase the global yield of the process from 28% to about 40%. Thus, this new procedure may be more suitable in order to get larger amounts of EMICORON for further preclinical studies.

Keywords: EMICORON, Organic Synthesis, Synthesis Improvements, G-quadruplex Structures, Anticancer Activity.

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1. Introduction

Polycyclic aromatic compounds with hydrophilic polar side chains are well known to inhibit telomerase [1] and thus have anticancer properties [2, 3]. In particular, modified perylene and coronene [4,5] derivatives have been shown to be highly selective with a good activity.

EMICORON represents a further development of the previous compounds. Indeed, it is a poly-aromatic compound constituted by constituted by a benzo[*ghi*]perylenic core that presents two ethyl-piperidine chains on the major axis plus one ethyl-piperidine chain and one piperidine chain on the minor axis (Figure 1)[6].

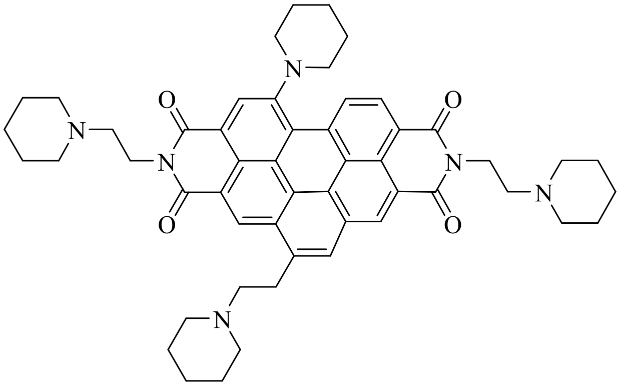

Figure 1: Structure of EMICORON

This compound was synthesized for the first time in our own laboratory in 2012 [6]. The first synthesis of EMICORON was carried out through a multistep linear synthesis including five steps [6, 7] (Figure 2). The first step which involves a bromination of the perylene-3,4:9,10-tetracarboxylic dianhydride, led to two regioisomers that are dibromoderivates of the initial anhydride [7]. Even though the regioselective synthesis of the desiderated regioisomer, the 1,7-dibromo-3,4:9,10-tetracarboxylic dianhydride (Figure 2, compound 2a) has already been performed [8], it is still more convenient, in terms of yields and atomic efficiency, to perform the bromination following the method reported by Franceschin and coworkers [7], and hence, to separate the obtained regioisomers by classic Column Cromatography (CC) only after the last step of the synthesis protocol. Yet, the yield of the global process was found to be only of 28%.

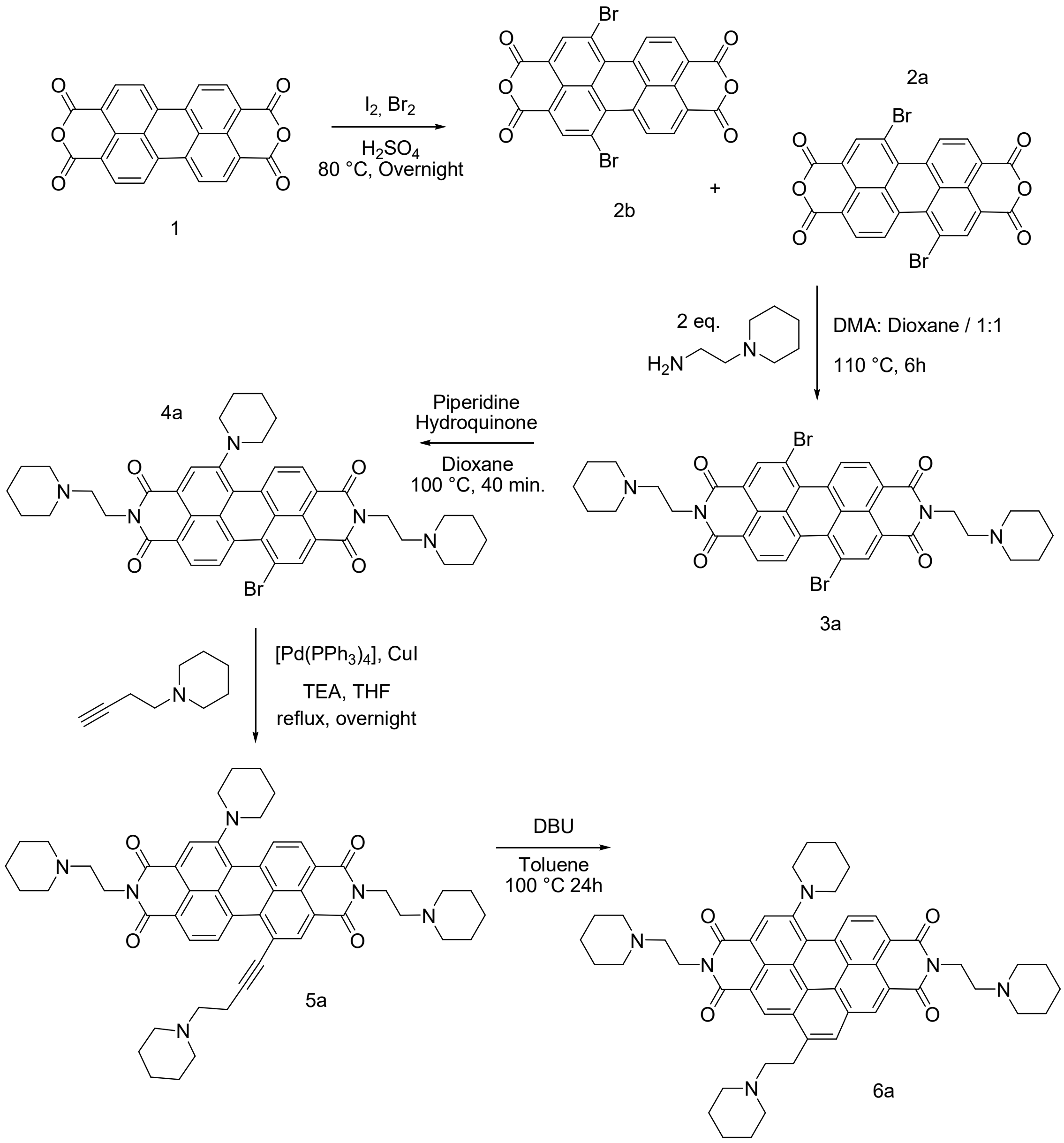

Figure 2: Schematic original protocol for the synthesis of EMICORON

Nevertheless, EMICORON has shown to exhibit in-vivo antitumor activity, being able to reduce the growth of colon-rectal cancer in rats [9]. This result has been shown to be achieved by a multi-target mechanism of action: at high doses it acts as a telomerase inhibitor [6]; at minor concentrations, it induces apoptosis in tumor cells by rapidly triggering extensive damage to telomeric DNA because of the displacement of the telomeric protein protection of telomeres 1 known as POT1 [6].

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EMICORON has also showed to bind G-rich oncogene promotors, down-regulating the expression of genes [10]. Moreover, in both in vitro and in vivo studies, it was observed that it acts with other conventional antitumor drugs in a synergic effect [11].

The aim and the main objective of this work were to improve the synthesis total yield in order to get more amounts of EMICORON for further preclinical studies.

2. Materials and methods

2.1. Materials

All the reagents, anhydrous solvents and deuterated solvents were purchased from Sigma-Aldrich and used with no further purification.

Silica gel for classical Column Chromatography (40-63 µm particle size) was purchased from Fluka Analytical, instead.

2.2. Instrumentation

^{1}H-NMR spectra were recorded on a Varian (now Agilent Technologies) Mercury 300 MHz spectrometer. $CDCl_3$ (deuteron-chloroform) was used as deuterated solvent and the chemical shifts were expressed from TMS (s, 0.00 ppm)

2.3. Synthesis

2.3.1 Synthesis of N,N'-Bis[2-(1-piperidino)-ethyl]-1,7-dibromoperylene-3,4:9,10-tetracarboxylic diimide (PIPER-Br) (3a)

Under argon atmosphere, 5.001 g of a mixture of the two isomers of dibromoperylene-3,4:9,10-tetracarboxylic dianhydride in ratio 8:1 (w/w) were dissolved at room temperature in 18 ml of anhydrous DMA and 18 ml of anhydrous dioxane. At this point, 2.6 ml of 1-(2 amminoetyl)-piperidine were added. The reaction mixture was stirred at 110 °C for 12 hours. After cooling, cold water (4 °C) was added. 6.522 g of the final product, actually a mixture of two isomers in ratio 8:1 (w/w), were obtained and isolated, by filtration, as a red solid. The yield was 93%.

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2.3.2 Synthesis of N,N'-Bis[2-(1-piperidino)-ethyl]-1-(1-piperidinyl)-7-bromoperylene-3,4:9,10-tetracarboxylic diimide (PIP-PIPER-Br) (4a)

Under argon atmosphere, 6.011 g of the products obtained from the previous reaction were dissolved in 30 ml of piperidine and stirred at 80 °C for 30 minutes. Cold water at 4 °C was added and the crude product was extracted with dicloromethane. Then, the organic phase was washed with water until the aqueous layer was neutral.

The product of interest, actually a mixture of two isomers in ratio 8:1 (w/w), for the weight of 4.502 g, with a yield of 71%, was isolated from the crude product, by Column Chromatography on silica gel, using a mixture of dichloromethane and methanol 95:5 (v/v) as eluting system.

2.4 NMR data of all the synthetic compounds

N,N'-Bis[2-(1-piperidino)-ethyl]-1,7-dibromoperylene-3,4:9,10-tetracarboxylic diimide (**3a**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.44 (2H, d, J = 8.1 Hz, aromatic H), 8.88 (2H, s, aromatic H), 8.66 (2H, d, J = 8.1 Hz, aromatic H), 4.36 (4H, t, J = 7.0 Hz, N_{imidic}-CH_2), 2.67 (4H, t, J = 7.0 Hz, $N_{piperidine}$-CH_2), 2.57-2.53 (8H, m, $CH_{2piperidine}$), 1.62-1.54 (8H, m, $CH_{2piperidine}$), 1.47-1.41 (4H, m, $CH_{2piperidine}$).

N,N'-Bis[2-(1-piperidino)-ethyl]-1-(1-piperidinyl)-7-bromoperylene-3,4:9,10-tetracarboxylic diimide (PIP-PIPER-Br) (**4a**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.41-9.34 (2H, m, aromatic H), 8.80 (1H, s, aromatic H), 8.57 (1H, d, J = 8.2 Hz, aromatic H), 8.49-8.46 (2H, m, aromatic H), 4.40-4.34 (4H, m, N_{imidic}-CH_2), 3.46-3.34 (2H, m, C_{ar}-$N_{piperidine}$-CH_2), 3.03-2.95 (2H, m, C_{ar}-$N_{piperidine}$-CH_2), 2.71-2.55 (12H, m, $N_{piperidine}$-CH_2 and $CH_{2piperidine}$), 1.89-1.42 (18H, m, $CH_{2piperidine}$).

3. Results and Discussions

The improvement in the synthesis of EMICORON was obtained by the modification of two reactions corresponding to the second and the third steps in the original protocol [6, 7].

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The former leads to the formation of N,N'-Bis[2-(1-piperidino)-ethyl]-1,7-dibromoperylene-3,4:9,10-tetracarboxylic diimide (PIPER-Br) (Figure 2, compound **3a**). With respect to the previous protocol, the reaction time was increased passing from 6 to 12 hours. Moreover, the precipitation of the product was performed by adding to the reaction mixture, water at 4 °C instead at room temperature (Figure 3). This new methodology was able to improve the yield of this reaction step of around 20%.

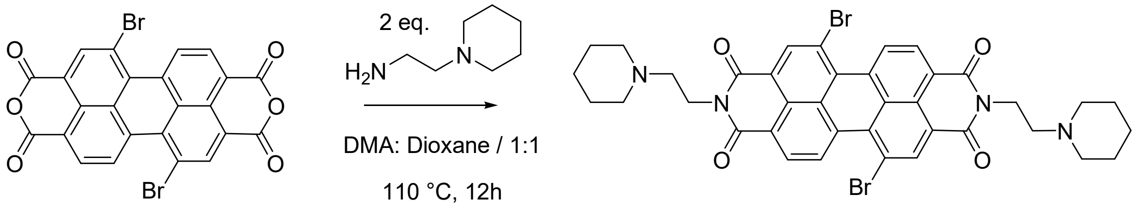

Figure 3: Scheme of the reaction modifications for step two

Indeed, the third step lead to the formation of PIP-PIPER-Br (N,N' Bis [2-(1-piperidino)-ethyl]-1-(1-piperidinyl)-7-bromoperylene-3,4:9,10-tetracarboxylic diimide (Figure 2, compound **4a**). The original protocol forecast the use of a mixture of dioxane and piperidine in ratio 1:1 (v/v) together with hydroquinone at 100 °C and for 40 minutes. The presence of hydroquinone resulted to be essential to promote the inhibition of some radical reactions which can happen in similar conditions leading to de-halogenated collateral products [12, 13]. Moreover, it was not possible to isolate the final product of this step as pure compound in such conditions and a further classical CC on silica gel was necessary in order to remove also some reaction products of the hydroquinone.

Indeed, the new protocol proceeded by totally removing dioxane, using piperidine both as solvent and reagent, and under an Argon atmosphere. In such conditions, the reaction was observed to yield in less time and at lower temperature (Figure 4).

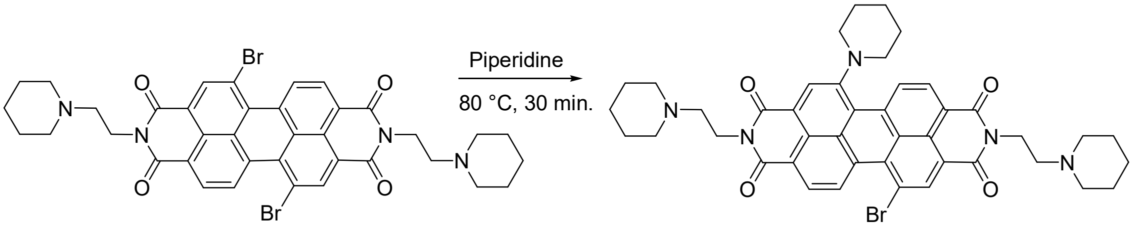

Figure 4: Scheme of the reaction modifications for step three

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The atomic efficiency of this reaction step was seen to be greatly increased this way and did not need a subsequent chromatographic step for the separations of the products and of the by-products. In fact, the derived products, the desired one (**4a**), its regioisomer and the two corresponding piperidinyl di-substituted compounds, can be directly used as starting material for the next synthesis step thus increasing the global yield for the synthesis of EMICORON.

4. Conclusions

The modification of the reaction conditions of two previously yield-limiting steps of the synthesis of EMICORON led to an increase of the global yield and avoided the use, in one case, of problematic solvents. Thus, this new protocol may really represent a new way to obtain EMICORON in higher amounts for medicinal purposes.

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