Communication

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Continuous-flow Sequential Schotten-Baumann Carbamoylation and Acetate Hydrolysis in the Synthesis of Capecitabine

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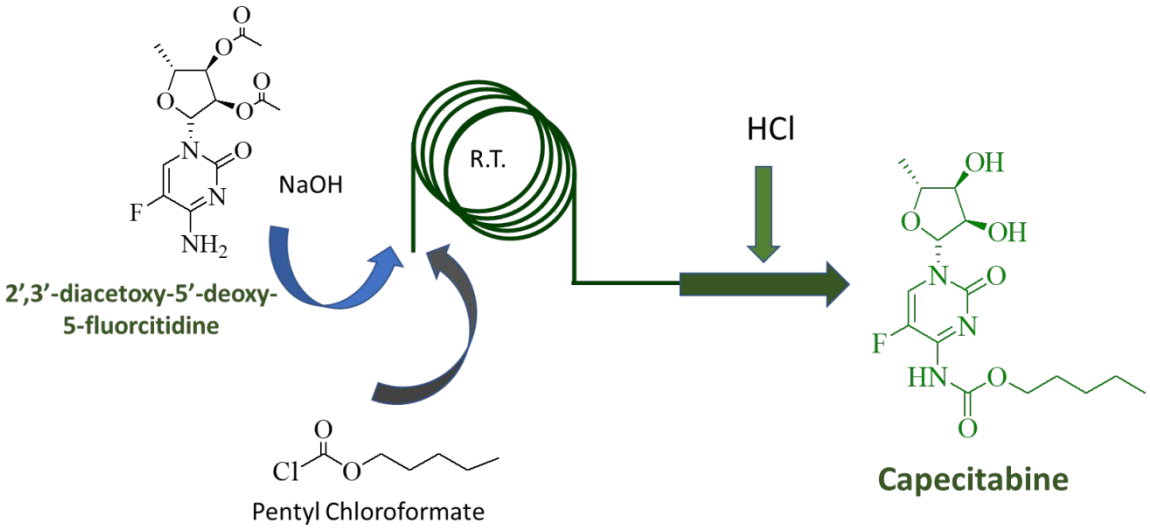


Abstract: Capecitabine is an important anti-cancer drug which synthesis comprises late stage carbamoylation and ester hydrolysis. Here in we report the use of Schotten-Baumann reaction in order to perform these transformation in one single pot, both in batch and continuous flow. Under batch Capecitabine was obtained in 82% yield in 5h while under continuos flow it was obtained in 81% in 30 minutes. This one pot reaction reduces the chemical waste produced, labor, time and cost and additionally comprises the use of environmentally friendly solvents and reagents as well as energy efficient and safe methods, all of which fulfill the requirements of a green process.

Key-Words: Capecitabine, Continuous-Flow, Carbamoylation, Schotten-Baumann Reaction

1-Introduction

Capecitabine **1** is a broad spectrum anti-cancer pro-drug of 5-fluorouracyl **2** used clinically as the first line treatment for metastatic colorectal cancer, as adjuvant in large bowel colon cancer and metastatic and advanced breast cancer[1,2]. 5-fluorouracil is an analogue of pyrimidine that acts as an antimetabolite, inhibiting the growth of tumor cells. In the body, capecitabine is transformed in the active 5-fluorouracyl through a sequence of enzymatic reactions. First, liver carboxyesterases hydrolase the carbamate functionality yielding 5'-deoxy-5-fluorocitidine **3**, which under the action of a cytidine deaminase, leads to the respective 5'-deoxy-5-fluoruridine **4.** Compound **4** then releases the toxic 5-fluorouracil in the tumor cells by the action of thymidine phosphorilase.[2] The release of the active anti-metabolite within the neoplasic cells greatly increases the safety of the drug.[3,4]

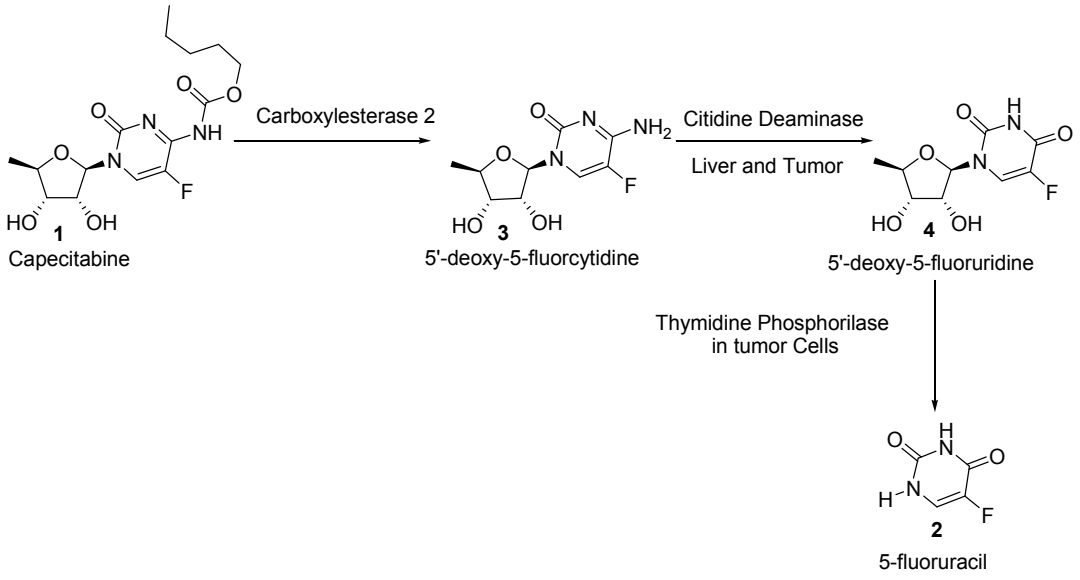
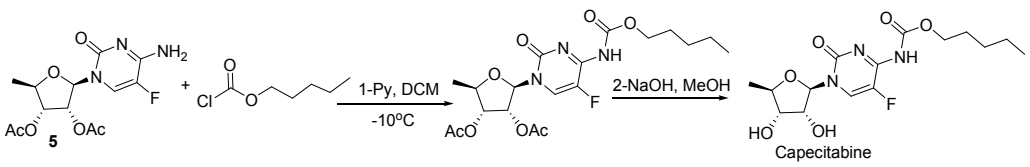


Figure 1: The release of the cytotoxic 5-fluorouracil from Capecitabine 1.

From the structural point of view, capecitabine is a pentyl carbamate derived from 5'-deoxy-5-fluorocitidine **3**. Over the literature, the large scale synthesis described for **1** [5-9], the carbamate group is introduced at a late stage, specifically on the

commercially available 2',3'-diacetoxy-5'-deoxy-5-fluorcitidine **5**. The carbamoylated nucleoside is then deprotected with the removal of acetyl groups from 2' and 3' positions, Scheme 1. These reported methods for carbamoylation/deprotection are laborious at large scale, once it demands the use of two different reactions to achieve the synthesis of the desired active pharmaceutical ingredient. Additionally, it also suffers from some drawbacks as the use of low temperatures and hazardous solvents such as pyridine, dichloromethane, dimethylformamide or toluene for carbamoylation and methanol for deprotection; all of which are inherently toxic class 2 solvents.



Scheme 1: Late stage carbamoylation of 2',3'-diacetoxy-5'-deoxy-5-fluorcitidine 5 in the course of capecitabine synthesis.

The Schotten-Baumann reaction is a method for the preparation of esters and amides with high potential to fulfill the principles for a green transformation which includes the use of environmentally friendly solvents and reagents as well as energy efficient methods.[10,11 12 13]. It is performed in water as solvent and in the presence of a base, usually an alkaline metal hydroxide or carbonate.[14] This transformation has also been used for the introduction of carbamate groups from chloroformates or anhydrides although to a much less extent then for the synthesis of amides or esters. The great advantage of such procedure is the use of safe green solvents and bases and, depending on the case, the drop in water solubility after the introduction of acyl group leads to a simple work up, a very important issue when dealing with the production of hazardous waste at large scale synthesis. Additionally, this reaction has already been studied under continuous-flow as exemplified by the synthesis of acyl-sulphonamide by White and co-

workers [15]. These advantages make the Schotten-Baumann reaction an interesting green method for the introduction of the pentyl-carbamate in the synthesis of capecitabine from the commercially available acetate **5**. In the present case, the Schotten-Baumann reaction can afford additional advantages once the use of aqueous base can lead to the carbamoylation-deprotection of the acetate groups in a one-pot reaction. Such "pot-economy"[16] is known to reduce the amount of chemical waste produced, labor, time and cost; increasing the efficiency and greenness of the process. However, such methodology does not come without any difficulties, where the presence of aqueous base may lead to over carbamoylation as well as to the competing hydrolysis of the pentyl chloroformate. Such issues can be addressed with the use of continuous flow conditions, once reactions conducted under continuous flow can present advantages over those conducted under batch conditions. The high surface-to-volume ratio enhance heat transfer and temperature control while the more efficient mixing in micro and mesoflow reactors (micromixing) allow better mass transfer.[17] [18] [19] Additionally, it improves the process in terms of accident prevention, which is also desirable in order to increase the greenness of any process.[20] [21] [22] [23]

The synthesis of capecitabine has already been reported under continuous-flow by Jamison and co-workers, where the glycosylation, carbamoylation and deprotection all occur in sequence, in three different compartments[24]. In their study, the carbamoylation is conducted in a mixture of acetonitrile/pyridine and the final ester deprotection with the introduction, in a different compartment, of a mixture of water/methanol, rendering a complex final reaction media. In our view, the development of a continuous-flow Schotten-Baumann reaction may allow carbamoylation and deprotection in water in one single reactor improving greenness and cost. In order to take advantage of these characteristics and our continuous efforts on enabling API

synthesis under continuous-flow conditions, here in we present our approach towards the continuous-flow carbamoylation-deprotection cascade under Schotten-Baumann conditions, bringing green chemistry in the context of capecitabine synthesis.

Results and Discussion

To the best of our knowledge, the Schotten-Baumann carbamoylation of capecitabine has never been reported on the literature. So, in order to gain information on the viability of such transformation, the reaction of 2',3'-diacetoxy-5'-deoxy-5-fluorcitidine (**5)** under batch conditions was carried out for the capecitabine synthesis. The reaction between pentyl chloroformate and **5** in a 10% NaOH aqueous solution afforded capecitabine in 42% isolated yield after 12h, with the concomitant persistent presence of the starting material. This result demonstrated the one-pot process feasibility for carbamoylation and acetate hydrolysis in the synthesis of capecitabine.

We hypothesized that this slow capecitabine formation was probably due to the immiscibility of the pentyl-chloroformate in the aqueous reaction media. In order to increase the rate of capecitabine formation, acetone was added as a co-solvent to the reaction mixture. In the presence of 20% of acetone, an increase in the rate of capecitabine formation was observed. After 5h of reaction at room temperature, capecitabine was isolated in 82% yield.

Interestingly, when this reaction was monitored by HPLC, it was observed the consumption of starting material 2',3'-diacetoxy-5'-deoxy-5-fluorcitidine (**5)**, with the concomitant formation of a substance with a retention time distinct from capecitabine. The reaction profile concerning the consumption of the staring material, this unknown intermediate and capectabine production is depicted in the graph present in Figure 2.

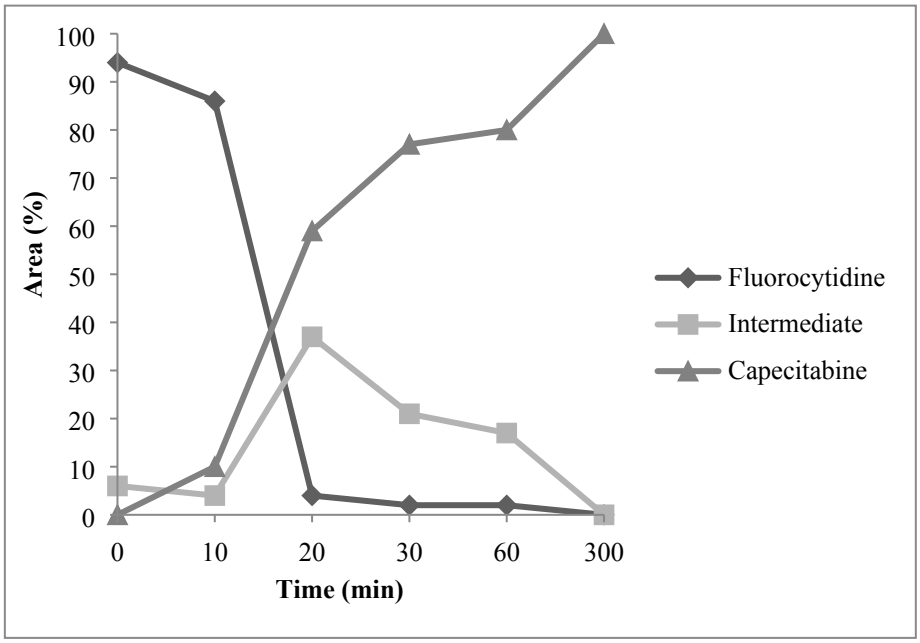
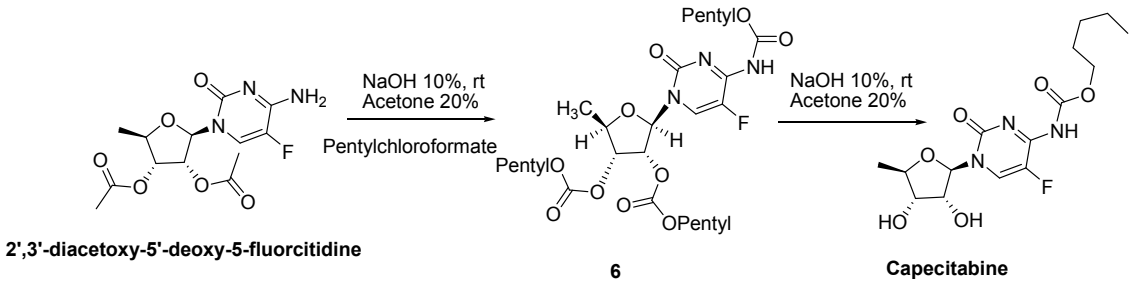


Figure 2: The reaction profile concerning the production and consumption of the staring material, intermediate and Capecitabine.

As can be seen in the graph, under the conditions of the Schotten-Baumann reaction in the presence of 20% acetone there is a fast consumption of the starting material with the concomitant formation of capecitabine and unknown intermediate, which slowly leads to the desired product.

This intermediate was isolated and analyzed by one and two dimensional $^{1}H,^{13}C$ NMR and by HRMS. The HRMS afforded a m/z ratio for its sodium adduct $[M+Na]^{+}$ of 610.2746Da, corresponding to a molecular formula of $C_{27}H_{42}FN_3O_{10}$, which is consistent with a compound resulting from the reaction of 5´-Deoxy-5-Fluorocytidine **5** with 3 equivalents of pentyl chloroformate (theoretical mass 610.2746 Da) [9]. Two dimensional NMR analysis (HMBC) show correlation between the carbonyl carbons with the 2' and 3' carbohydrate hydrogens suggesting that the unknown substance to be

compound **6**, instead of possible isobaric imide [8] (see Supporting Information). The presence of this product early in the reaction demonstrates a rapid hydrolysis of the acetates present at positions 2' and 3' and their subsequent reaction with the pentyl chloroformate. A separate reaction where **6** is submitted to the same condition of the carbamoylation demonstrate that it affords capecitabine in high yields.



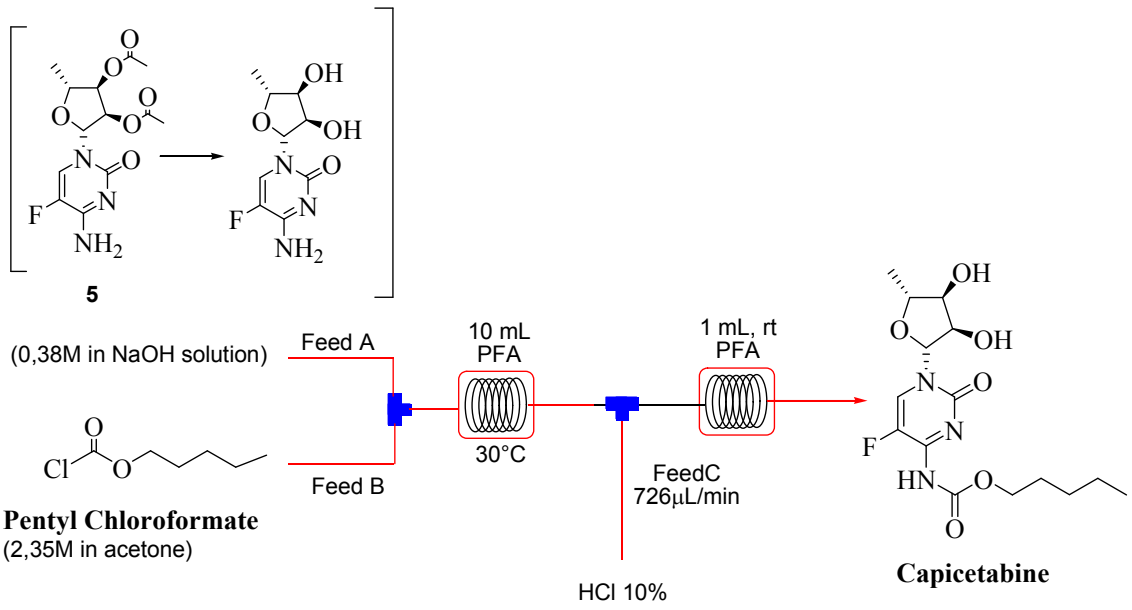
Scheme 2: The sequence producing the intermediate and capecitabine.

Importantly to note that intermediate **6** has already been reported previously by researches from Hoffmann-La Roche. [25] In this report, the reaction of the 5'-deoxy-5-fluorcytidine with penthyl-chloroformate was carried out in pyridine and dichloromethane at -10°C. In this patent, capectabine is delivered in a subsequent reaction with sodium hydroxide in methanol also at -10°C. The results presented in Figures 1 and 2 demonstrates that the sequential acetate hydrolysis, carbamoylation and hydrolysis of the carbonates at 2' and 3' positions can be carried out as an one-pot reaction using only water:acetone as solvent and sodium hydroxide as base at room temperature in high yield.

With the feasibility of the Schotten-Baumann reaction demonstrated in the synthesis of capecitabine, we moved to its optimization under continuous-flow. In order to evaluate the effect of temperature and base concentration on the reaction, an optimization was run in a dedicated microwave reactor, and then translated and refined to the continuous-flow processes, as depicted in table 1.[26] The translation of the

optimized reaction conditions to the continuous flow was conducted with two different feeds using two Asia flow chemistry syringe pump and a 10mL Teflon tubing with 0.125'' OD and 0.00625'' I.D.

Table 1: Continuos-Flow reaction between 2',3'-diacetoxy-5'-deoxy-5-fluorcitidine **5 and Pentyl Chloroformate**



Entry	Residence time (min)	Feed A (µL/min)	Feed B (µL/min)	NaOH % (m/v)	Capecitabine (%)
1	15	484	182	40	72
2	30	242	90.8	40	81
3	30	242	90.8	30	41
4	30	242	90.8	20	29
5	30	242	90.8	10	0
6	60	121	45.5	40	48
7	60	242	90.8	30	8

Different base concentrations and residence times were evaluated. Both variables had a profound impact on the reaction conversion. The highest yield of capecitabine was observed with 30 minutes of residence time with the use of a NaOH 40% solution (entry 2) at 30°C, where higher temperature lead to lower yields due to decomposition

of the starting material (See Supporting Information). Lower yields were observed with shorter residence time due to incomplete reaction (entry 1 vs entry 2) as well as lower base concentration. Interestingly, longer residence times produced, with the same base concentrations (entries 2 and 6 as well as 3 and 7), lower the yields of capecitabine due to decomposition.

In order to verify the robustness of the methodology developed, a steady state experiment was performed and was observed that the system was stable at constant conversion for a period higher then 4 hours as shown in figure 3.

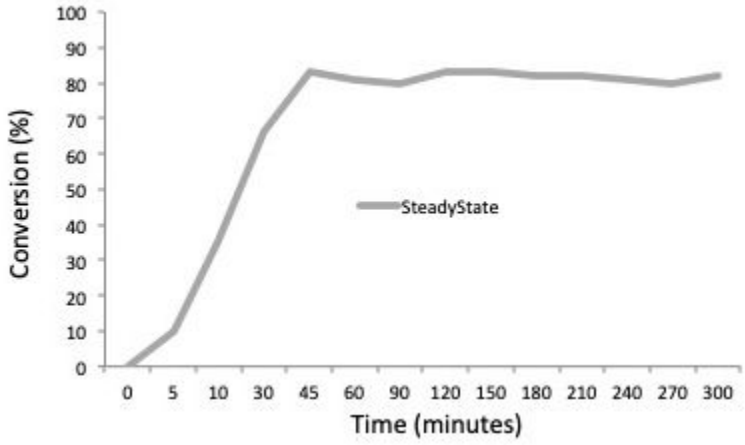


Figure 3: The Robustness of the developed synthesis of Capecitabine under continuous-flow.

An important observation was made during the continuous-flow reaction. It was observed that the dissolution of **5** in the 40% NaOH solution, which leads to the higher yields of Capecitabine (entry 2), rapidly hydrolyze the 2', 3'acetate groups, leading, in fact, to the pumping of 5′-Deoxy-5-Fluorocytidine. This is an interesting observation since no intermediate could be observed under continuous flow. The immiscibility of the NaOH 40% and acetone solutions and the increased nucleophilicity of the nitrogen atom may prevent over acylation to the production of **6**.

Conclusion:

The use of the Schotten-Baumann reaction for the introduction of the pentyl-carbamate in the synthesis of Capecitabine from 2',3'-diacetoxy-5'-deoxy-5-fluorcitidine resulted in a high yield (82%) green one-pot reaction where the carbamoylation and deprotection reactions occurred in a one-pot process. This "pot-economy" enabled the development of a transformation without the use of the hazardous solvents reported in batch as well as in flow, and with the avoidance of low-temperature, intermediate work-up and isolation, thus decreasing cost, time and labor. When this reaction was translated to continuous-flow synthesis of capecitabine the reaction time could be drastically decreased leading to a clean one-pot reaction in only 30 minutes in 81% isolated yield.

Experimental Section:

Schotten-Baumann Carbamoylation and Deprotection of 5: In a 20 mL reactor warmed at 30 ºC, was added a solution of 2',3'-diacetoxy-5'-deoxy-5-fluorcitidine (3.04 mmols) in 8 mL of 10% NaOH. To this mixture was added dropwise a solution of n-pentyl chloroformate (7.06 mmols; 2.41 equiv.) in acetone (2 mL). After 5h, the reaction medium was acidified with 10% HCl (10 mL) to pH 4 and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over sodium sulfate, filtered and the solvent removed under reduced pressure. The crude thick yellow oil was crystallized from hexane to give a white crystalline solid with the same chromatographic and spectroscopic data of capecitabine in 82% yield.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (s, 1H), 5.65 – 5.64 (m, 1H), 4.21 – 4.20 (m, 2H), 4.14 – 4.09 (m, 2H), 3.82 – 3.79 (m, 1H), 1.65 – 1.60 (m, 2H), 1.35 (d, J = 6.4 Hz, 3H), 1.31 – 1.27 (m, 4H), 0.84 (t, J = 6.8 Hz, 3H). δ ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.40, 153.29, 92.17, 80.83, 75.06, 66.89, 28.30, 27.93, 22.36, 18.67, 13.99.

Synthesis of intermediate 6: The same experimental protocol described for Carbamoylation/deprotection of **5** was employed. However, in this case the reaction was interrupted after 1h. After the usual workup a crude solid was obtained, which was purified by chromatography in sílica gel (20% AcOEt in hexane) to furnish a white solid (35% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 11.95 (s, 1H), 7.34 (s, 1H), 5.79 (d, J = 3.6 Hz, 1H), 5.23 (s, 1H), 4.85 (s, 1H), 4.69 (s, 1H), 4.28 – 4.19 (m, 1H), 4.07 (dd, J = 12.7, 6.1 Hz, 6H), 3.98 (t, J = 6.7 Hz, 1H), 1.69 – 1.52 (m, 7H), 1.41 (d, J = 6.2 Hz, 3H), 1.34 – 1.21 (m, 14H), 0.83 (s, 10H).^{13}C NMR (101 MHz, $CDCl_3$) δ 153.07, 153.03, 152.40, 152.22, 68.26, 68.07, 65.67, 64.26, 27.58, 27.22, 27.17, 27.12, 26.93, 26.91, 26.68, 26.64, 21.32, 21.30, 21.22, 17.31, 16.12, 12.96, 12.93, 12.88. HRMS $[M+Na]^+$ of 610.2746 Da

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Supporting Information: All experimental details, Chromatographic and Spectroscopic data are presented in the Supporting Information File

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