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*Valentina Mancino, Bruno Cerra, Alessandro Piccinno, Antimo Gioiello**

Laboratory of Medicinal and Advanced Synthetic Chemistry, Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, 06122 Perugia (Italy).

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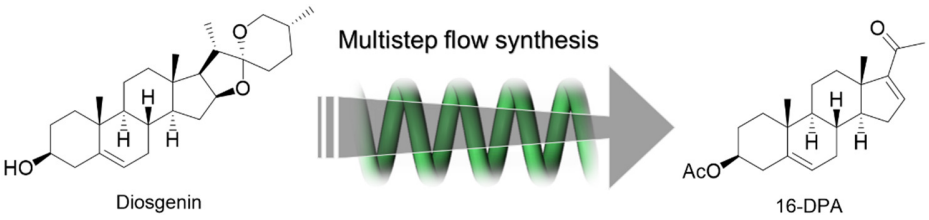


KEYWORDS: Steroids, flow chemistry, continuous flow processing, in-line work-up, telescoped process.

Abstract

A telescoped multi-step process to provide the continuous delivery of 16-dehydropregnenolone acetate (16-DPA) from diosgenin is described. The method has been evaluated through batch screenings helpful to identify critical bottlenecks and flowability, and the best conditions have been optimized in flow systems before telescoping the individual steps together in a single, integrated flow process. Further highlights of our approach include the use of efficient in-line extraction operations and reaction monitoring, the avoidance of time-consuming purifications between steps as well as improvement of efficiency and safety standards.

1. Introduction

The most significant expansions of steroid chemistry came with the discovery of cortisone and progesterone as important therapeutic agents.[1] Indeed, to make them available as marketed drugs, chemists had to surmount a substantial obstacle, i.e. the synthetic accessibility. This led to the so-called "golden age of steroids" occurred in the 50's and beyond, when an unusually large number of the world-leading chemists was attracted to the field to render steroids available for clinic and drug production.1 Nowadays, the global market of steroidal drugs and intermediates is estimated to be over a billion dollars preserving the interest in developing new methods for steroid synthesis. Novel strategies and improved protocols of well-established methods are therefore highly desired to meet modern synthesis criteria such as sustainability, safety, and cost. To this aim, a major impetus came with the adoption of technological solutions as flow processing.[2] However, while many chemical practices have already benefited from flow chemistry, assessment of the literature indicated steroid manipulation to be poorly explored in flow systems.[3] Furthermore, to the best of our knowledge, only one recent example of two step synthesis of steroids under flow conditions has been reported so far and relates with the production of ursodeoxycholic acid using immobilized enzymes.[4]

In this work, we were challenged to develop a continuous flow process for the preparation of 16-dehydropregnenolone acetate (16-DPA) (**1**), a key substrate for the production of several natural steroids, sex hormones, and drugs (Figure 1).[5] It is worth noting that most of steroidal drugs are prepared from 16-DPA (**1**) with few of which obtained by total synthesis.[6]

The overall process we pursued to investigate started from diosgenin (22-iso-5-spirostane-3β-ol) (**2**), a cheap and readily available plant-derived sapogenin obtained by extraction from *Dioscorea floribunda* (Scheme 1).[7] The method proceeded through (a) acetylation/acetolysis

reaction of **2** to form the furostenol derivative namely pseudodiosgenin-3,26-diacetate (**3**), (b) oxidative cleavage of the $\Delta^{20\text{-}22}$ double bond to get diosone (**4**) that was readily submitted to (c) hydrolysis followed by elimination to yield the final product **1**.

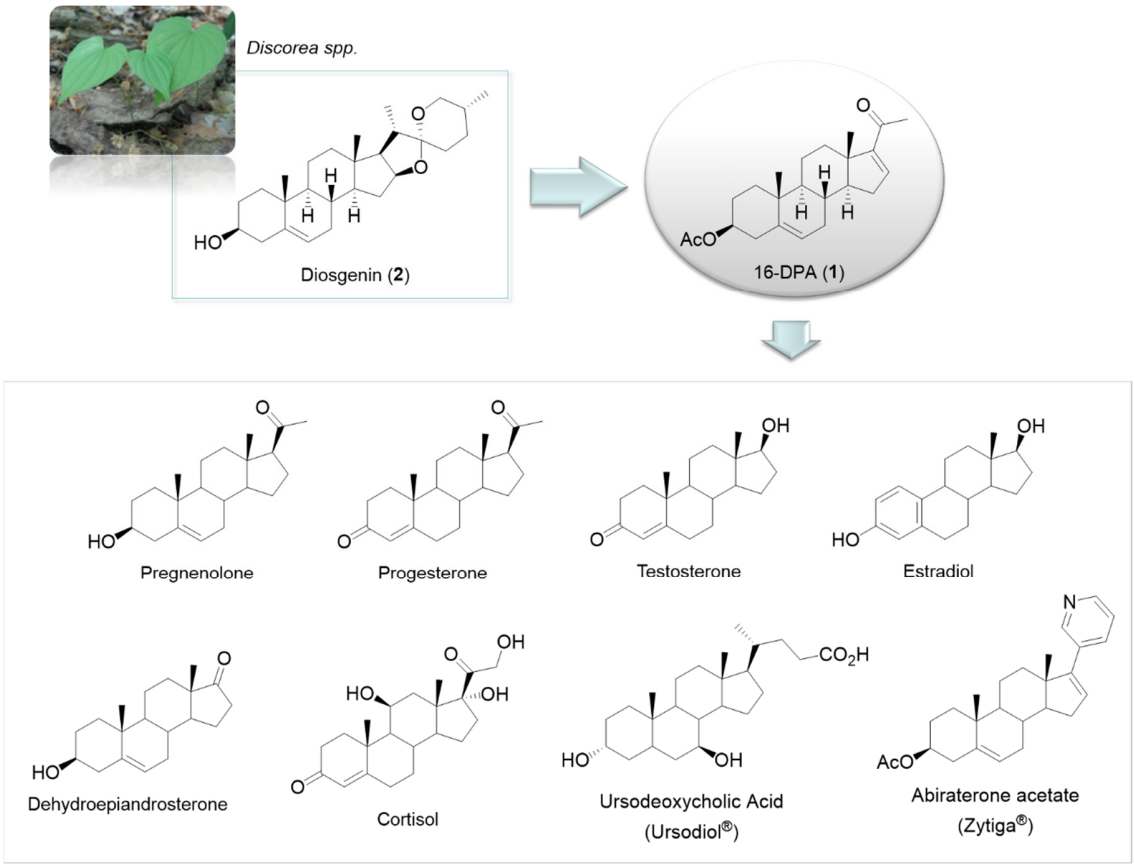
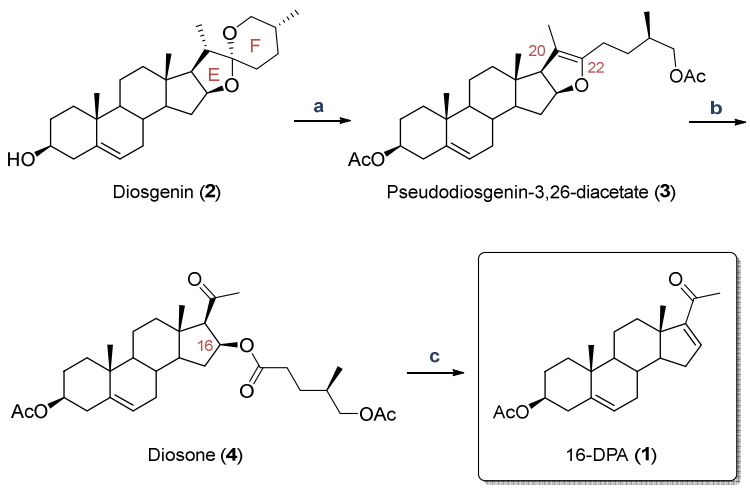


Figure 1. 16-Dehydropregnenolone acetate (**1**), a crucial synthetic precursor of natural steroids and drugs.

Marker's degradation is the first known route for making 16-DPA (**1**) from diosgenin (**2**) (Scheme 1).[8] The method consisted in refluxing diosgenin (**2**) in an autoclave with acetic anhydride (Ac_2O) in xylene for 14 h at 5 bar pressure and the resulting pseudodiosgenin **3** was then oxidized with CrO_3 and hydrolysed under acidic conditions to get 16-DPA (**1**) in 60% overall yield.

Nowadays, diverse protocols are available for batch synthesis (Table S4, Supporting Informations),[9] and although major advances have been made, further criteria need to be met for modern manufacturing process. For instance, the first step of acetolysis generally requires harsh reaction conditions as elevated temperature and pressure that strongly affect the sustainability of the process and the quality of the end product. Moreover, the high temperature required to promote the reaction combined with the non-homogenous batch heating is potential cause of side products formation, unsatisfactory yields, and the need for tedious purifications. The following oxidation step also needs improvements to avoid the use of hazardous reagents and expensive (co)oxidant that raise issues in terms of safety, waste production and costs. To this end, other groups reported the use of various additives like pyridine or the employment of high boiling solvents.[8c-e,g,h] A recent synthesis of 16-DPA (**1**) was realized by heating diosgenin (**2**) in xylene in presence of smaller amounts of Ac_2O (3.5 equiv.) at 250 °C for 10 h to give pseudodiosgenin-3,26-diacetate (**3**) (91%) that was then reacted with CrO_3 in acetic acid at -10/10 °C under ultrasound irradiation for 1 h.[8c] 16-DPA was obtained in 57% yield by refluxing diosone (**4**) in acetic acid for 2 h. Despite the advantages with respect to previous methods, the use of high temperatures, long reaction time and the difficult control and tuning of ultrasound reaction conditions, make this strategy not ideal for commercial production and hence alternative methods are needed.



Scheme 1. General route for converting diosgenin (**2**) into 16-dehydropregnenolone acetate (**1**).

Consequently, we aim to develop an integrated flow process that would overcome such limitations and adhere to modern synthesis principles. We initially proceeded with the evaluation of the process through batch screenings helpful to identify critical bottlenecks, flowability issues and best conditions before attempting to telescope the individual reactions together in a single, integrated flow process.

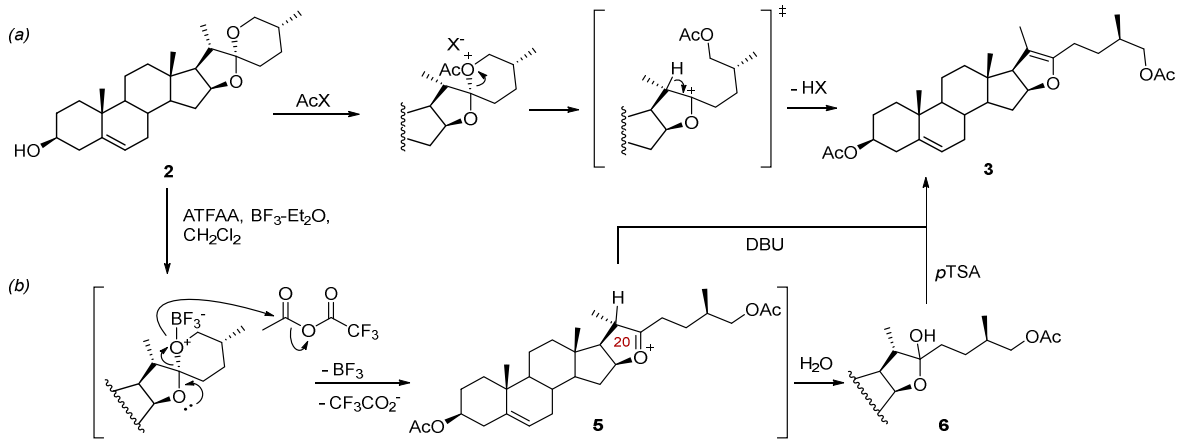
2. Results and discussion

2.1 From batch to flow

Step a: acetylation/acetolysis of diosgenin

The acetylation/acetolysis step converting diosgenin (**2**) into pseudodiosgenin-3,26-diacetate (**3**) proceeds according to the mechanism depicted in Scheme 2. We previously argued that the isomerization of **2** into **3** generally required high temperature and pressure, which may lead to extensive decomposition of the starting material and side products formation.[8c] Considering this fact, we had the idea of combining the potential of microwave irradiation with flow technology.[10]

Unfortunately, although batch screens in neat conditions gave high yields (Table S1, Supporting Information), preliminary attempts to translate the protocol under flow conditions (Figure S1, Supporting Information) were negative because of system overpressure. The employment of different solvent systems (THF, EtOAc/MEK, THF/MEK) and Lewis acids (0.1 equiv.) were also unsuccessful (Table S2, Supporting Information). The only exceptions were the reactions conducted with $AlCl_3$ and $BF_3{\cdot}Et_2O$ that furnished **3** in 30% and 40% yield, respectively. Overall, these observations prompted us to investigate a different strategy.



Scheme 2. One-pot acetylation/acetolysis mechanism for the formation of pseudodiosgenin-3,26-diacetate (**3**) from diosgenin (**2**) with (B) or without $BF_3{\cdot}Et_2O$ (A).[8i;11]

With the desire of promoting the isomerization of **2** into **3** at room temperature, we run a series of explorative reactions by employing the more reactive acetic trifluoroacetic anhydride (ATFAA)[10] as the acetyl source in the presence of a stoichiometric amount of $BF_3{\cdot}Et_2O$ that was preferable to $AlCl_3$ as it did not generate insoluble salts. ATFAA was freshly prepared by slow addition of AcOH (1.2 equiv.) to trifluoroacetic anhydride (TFAA) (1.0 equiv.) maintained at room

temperature in dark conditions and stirring for 5 min. The reaction of **2** with ATFAA and $BF_3{\cdot}Et_2O$ in CH_2Cl_2 at room temperature gave intermediate **5** that was then converted into **3** by means of 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) or *p*-toluenesulfonic acid (*p*TSA)[11] employed as CH_2Cl_2 soluble base and acid (Scheme 2b). Interestingly, DBU was significantly more efficient than *p*TSA (37% yield) affording the desired compound **3** in 90% yield.[a]

At this stage, the reaction with DBU was repeated in flow mode using different set-ups (Table 1). Thus, reagents were fed by loop injection, using two pumps dedicated to the organic solvent (CH_2Cl_2), a 10 mL PTFE coil reactor, a BPR (100 psi), an UV detector and a fraction collector (Figure 2). After the loop injection of the solution A and B, and the switching of the valves to the loops, the two solutions were mixed in a T-junction, and pumped through the coil reactor at room temperature. Initially, the outflowing mixture was directly quenched onto DBU (16.0 equiv.) in semi-batch modality (Table 1, entries 1-4). Subsequently, applying a different set-up, a CH_2Cl_2 stream containing DBU (0.96 M, flow rate= 2 mL/min) was inserted at the output line of the reactor and the outflow was collected using a fraction collector (Figure 2). The conversion and reaction yield were determined by ^{1}H-NMR analysis of the crude mixtures. This approach was suitable to screen different system set-ups using the minimum amount of the starting material **2**. The presence of the injection loop system and the connection with a fraction collector allowed to process samples of **2** in succession using the same solvent and varying the experimental conditions. The in-line UV detector was instrumental to monitor and detect reactions and related components. Thus, the

[a] Reactions were performed on 100 mg of **2** (0.24 mmol) using freshly prepared ATFAA (11.0 equiv.) and $BF_3{\cdot}Et_2O$ (2.0 equiv.) in dry CH_2Cl_2 (10 min, r.t.), and then adding DBU or *p*TSA. The relative yield was determined by ^{1}H-NMR analysis of the crude reaction mixture.

absence of the UV signals from the collected output indicated the end of the reaction and the possibility to start the next experiment after line washing with CH_2Cl_2.

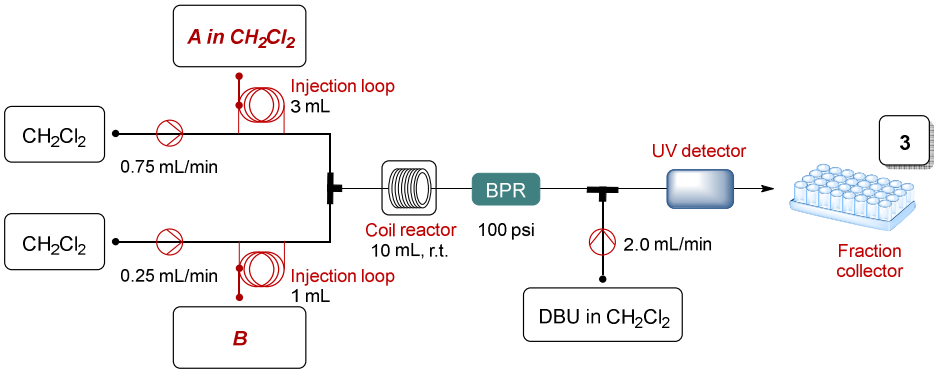


Figure 2. Flow set-up employed for exploring flow set-up and reaction optimization (Tables 1 and 2).

As a result, a complex reaction mixture was obtained when a 0.15 M solution of diosgenin (**2**) in CH_2Cl_2 was reacted with ATFAA and $BF_3{\cdot}Et_2O$ (Table 1, entries 1 and 2), while when ATFAA was flowed as the sole reaction component (pump B) a better reaction outcome was achieved (Table 1, entries 3 and 4). The best yield was reached using the conditions reported in Table 1, entry 5: in this case, a solution of DBU in CH_2Cl_2 was pumped *via* syringe pump, mixed with the reaction crude in a T-junction, the outflow was quenched with H_2O, and the organic phase was separated from the aqueous waste to afford **3** in 75% yield. Importantly, we also found that DBU was not necessary to promote the conversion of **5** into **3** under flow conditions (Table 1, entry 6).

Table 1. Different set-up for the conversion of diosgenin (**2**) into pseudodiosgenin-3,26-diacetate (**3**) under flow conditions.[a]

Entry	A	B	Conversion yield[b]	Yield[b]
1	**2**	ATFAA, $BF_3{\cdot}Et_2O$	Quantitative	(Complex mixture)
2	**2**, ATFAA	$BF_3{\cdot}Et_2O$ (0.96 M in CH_2Cl_2)	90%	(Complex mixture)
3	**2**, $BF_3{\cdot}Et_2O$	ATFAA	Quantitative	60%
4[c]	**2**, $BF_3{\cdot}Et_2O$	ATFAA	Quantitative	50%
5[d]	**2**, $BF_3{\cdot}Et_2O$	ATFAA	Quantitative	75%
6[e]	**2**, $BF_3{\cdot}Et_2O$	ATFAA	Quantitative	75%

[a] Reactions performed on 200 mg of **2** (0.48 mmol) using the flow set-up depicted in Figure 2. *Reagents and conditions*: **2** 0.15 M in dry CH_2Cl_2, ATFAA (11.0 equiv.), $BF_3{\cdot}Et_2O$ (2.0 equiv.), τ = 10 min, r.t., then DBU (16.0 equiv.). [b] Determined as **2** consumption by ^{1}H-NMR analysis of the crude reaction mixture. [c] DBU was maintained under magnetic stirring. [d] DBU (0.96 M) in CH_2Cl_2 was pumped *via* syringe pump (flow rate = 2 mL/min) and mixed with the reaction mixture in a T-junction. Then the mixture was poured into H_2O and the phases separated. [e] Reaction performed without DBU.

We next decided to test the effect of (higher) concentration and flow rate to enhance productivity, as well as the use of lower amount of $BF_3{\cdot}Et_2O$ and ATFAA to reduce cost and waste generation. Since a better solubility of diosgenin (**2**) in CH_2Cl_2 was observed in the presence of $BF_3{\cdot}Et_2O$, the reaction was conducted at 0.23 M of **2** that represented the maximum concentration ensuring solubility. While these conditions and the halving of the residence time gave positive outcomes (Table 2, entries 1 and 4), the reduction of $BF_3{\cdot}Et_2O$ and ATFAA equiv. was not relevant (Table 2, entries 2 and3). It is worth noting that when **2** was mixed with 1.1 equiv. of $BF_3{\cdot}Et_2O$ at the concentration of 0.23 M, the formation of a suspension was observed. Therefore, the best option turned out to be treating a solution of **2** (0.23 M) and $BF_3{\cdot}Et_2O$ (2.0 equiv.) in CH_2Cl_2 with freshly prepared ATFAA (11.0 equiv.) at room temperature with a residence time of 10 min.

Table 2. Optimization of the conditions for the flow synthesis of pseudodiosgenin-3,26-diacetate (**3**) from diosgenin (**2**) in flow modality.[a]

Entry	Concentration of 2	$BF_3{\cdot}Et_2O$ equiv.	ATFAA equiv.	Residence time	Yield[b]
1	0.23 M	2.0	11.0	10 min	80%
2	0.15 M	1.1	11.0	10 min	75%
3	0.15 M	2.0	5.5	10 min	68%
4	0.15 M	2.0	11.0	5 min	79%

[a] Reactions performed on 200 mg of **2** (0.48 mmol) using the flow set-up depicted in Figure 2. *Reagents and conditions*: **2** 0.23 or 0.15 M in dry CH_2Cl_2, ATFAA (5.5 or 11.0 equiv.), $BF_3{\cdot}Et_2O$ (1.1 or 2.0 equiv.), τ = 5 or 10 min, r.t. [b] Determined by ^{1}H-NMR analysis of the crude reaction mixture.

Step b: Oxidative cleavage of pseudodiosgenin-3,26-diacetate to diosone

The necessity of removing drawbacks for the oxidative cleavage of pseudodiosgenin-3,26-diacetate (**3**) to form diosone (**4**) (Scheme 1, step b) prompted us to evaluate different protocols starting from a preliminary batch screen. In the past, we and others found the use of H_2O_2 to be beneficial for eco-friendly flow oxidations.[12] In line with these findings, we tested the efficacy of H_2O_2 as co-oxidant in the presence of different metal oxides employed as oxidants in substoichiometric amount (Table 3). The best result was obtained using a catalytic amount of OsO_4 (0.01 equiv.) in CH_2Cl_2/DMF that furnished the desired diosone (**4**) in 70% yield after 30 min at room temperature (Table 3, entry 7). While we found a novel procedure amenable for flow applications, the inherent high toxicity of OsO_4 led us to proceed with the investigation of diverse oxidant systems based on the use of $KMnO_4$ and CrO_3.[13]

Table 3. Oxidative cleavage of pseudodiosgenin-3,26-diacetate (**3**) to diosone (**4**) with H_2O_2 and metal oxides in batch conditions at room temperature.[a]

Entry	Reagents (equiv.)	Reaction conditions	Yield[b]
1[14]	H_2O_2 (30% w/w) (2.0 equiv.)	AcOH, ultrasound, 1 h	(Complex mixture)
2	H_2O_2 (30% w/w) (4.0 equiv.), CrO_3 (0.1 equiv.)	AcOH, 1 h	(Complex mixture)
3	H_2O_2 (30% w/w) (4.3 equiv.) CrO_3 (0.08 equiv.)	THF, 24 h	(Complex mixture)
4	H_2O_2 (30% w/w) (4.3 equiv.) SeO_2 (0.08 equiv.)	THF, 3 h	(Complex mixture)
5	H_2O_2 (30% w/w) (4.3 equiv.) SeO_2 (0.08 equiv.) $NaIO_4$ (1.5 equiv.)	THF, 3 h	(Complex mixture)
6	H_2O_2 (30% w/w) (4.3 equiv.) SeO_2 (0.08 equiv.), then $NaIO_4$ (1.5 equiv.)	THF, 3 h	(Complex mixture)
7	H_2O_2 (30% w/w) (4.3 equiv.) V_2O_5 (0.08 equiv.) AcOH (2.0 equiv.)	THF, 3 h	40%
8	H_2O_2 (30% w/w) (4.3 equiv.) $VO(acac)_2$ (0.08 equiv.)	THF, 3 h	(Complex mixture)
9	H_2O_2 (30% w/w) (4.3 equiv.) $VO(acac)_2$ (0.08 equiv.) AcOH (2.0 equiv.)	THF, 3 h	(Complex mixture)
10[15]	H_2O_2 (30% w/w) (4.0 equiv.) OsO_4 (0.01 equiv.) DMF (20.0 equiv.)	CH_2Cl_2, 30 min	70%

[a] Reaction performed on 100 mg of **3** (0.20 mmol). *Reagents and conditions*: H_2O_2 (30% w/w), oxidant, solvent. [b] Determined by ^{1}H-NMR analysis of the crude reaction mixture.

A recent work proposed the use of $KMnO_4$ (5% mol) in the presence of $NaIO_4$ (5% mol) and triethyl benzyl ammonium bromide (TEBA) (2.2% mol) to synthesize diosone (**4**) from **3**.[8a]. With the perspective to use the crude solution of **3** deriving from the previous step, we run a set of experiments using $KMnO_4/NaIO_4$ as oxidizing system and CH_2Cl_2 as the organic solvent of choice (Table S3, Supporting Information). In all cases, complex mixtures were obtained. Remarkably, when $KMnO_4$ was replaced with CrO_3 (1.4 equiv.) in a monophasic solvent system constituted by CH_2Cl_2/H_2O/AcOH (1:1:0.5, v/v/v), **4** was obtained in 80% yield (Table S3). These last conditions were translated and further optimized into a flow apparatus using the set-up depicted in Figure 3. Our scope was to evaluate the efficiency of the oxidative species in different solvent systems either in the presence or not of a phase transfer agent or a co-solvent (Table 4).

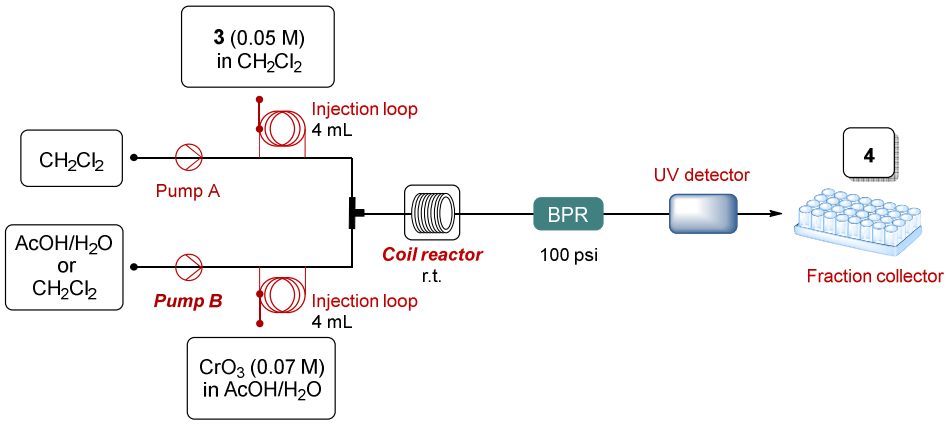


Figure 3. Flow set-up used for the optimization of the reaction conditions reported in Table 4.

Table 4. E-ring oxidative cleavage of pseudodiosgenin-3,26-diacetate (**3**) to diosone (**4**) under flow conditions.[a]

Entry	Flow rate (mL/min)	Phase transfer/ co-solvent (Pump B)	Reactor volume (mL)	Conversion yield[b]	Yield[b]
1	0.2	-	10	-	-
2	0.4	-	20	-	-
3	0.4	TEAI (0.02 equiv.)	20	15%	10%
4	0.4	TEAI (0.10 equiv.)	20	Quantitative	10%
5	2.0	TEAI (0.02 equiv.)	20	-	-
6	0.4	TEAI (0.02 equiv.)	20[c]	26%	20%
7	0.2	THF (5% v/v)	10[c]	-	-
8	0.2	MeCN (5% v/v)	10[c]	-	-

[a] Reaction performed on 100 mg of **3** (0.20 mmol) using the flow set-up reported in Figure 3. *Reagents and conditions*: **3** 0.05 M in CH_2Cl_2, CrO_3 (1.4 equiv.) 0.07 M in AcOH/H_2O (1:2, v/v), solvent B: H_2O, phase transfer agent or co-solvent, r.t. [b] Determined as **3** consumption by ^{1}H-NMR analysis of the crude reaction mixture. [c] Reactor immersed in ultrasound bath.

Unfortunately, only low yields were obtained applying different residence times, either using or not tetraethylammonium iodide (TEAI, 0.02 or 0.10 equiv.) (Table 4, entries 1-6). Also the employment of co-solvents as THF and MeCN was not beneficial for the reaction (Table 4, entries 7 and 8). Therefore, the CrO_3-promoted oxidative cleavage was studied in different batch monophasic systems (Table 5). Thus, a solution of **3** in AcOH was reacted with a AcOH/H_2O (9:1, v/v) solution of CrO_3 (1.4 equiv.), yielding 90% of **4** in 5 min (Table 5, entry 1). Remarkably, the same result was attained when the starting material **3** was dissolved in CH_2Cl_2 (Table 5, entry 2).

In an attempt to reduce the equiv. of CrO_3, the reaction was repeated with a lower amount of oxidizing agent (1.1 equiv.) (Table 5, entry 3). Sub-stoichiometric amounts of oxidant (0.1 or 0.5 equiv.) were used in the presence of *N*-methylmorpholine-*N*-oxide (NMO, 3.0 equiv.) resulting in complex reaction mixtures and unreacted **3** (Table 7, entries 4-6).

Table 5. E-ring oxidative cleavage of pseudodiosgenin-3,26-diacetate (**3**) to diosone (**4**) with stoichiometric CrO_3 or catalytic CrO_3 and NMO in batch conditions.[a]

Entry	Reagents	Solvent and conditions	Conversion yield[b]	Yield[b]
1	CrO_3 (1.4 equiv.) (in AcOH/H_2O 9:1, v/v)	AcOH, 5 min	Quantitative	90%
2	CrO_3 (1.4 equiv.) (in AcOH/H_2O 9:1, v/v)	CH_2Cl_2, 5 min	Quantitative	90%
3	CrO_3 (1.1 equiv.) (in AcOH/H_2O 9:1, v/v)	CH_2Cl_2, 5 min	85%	80%
4	CrO_3 (0.1 equiv.) NMO (3 equiv.)	CH_2Cl_2, AcOH/H_2O (9:1 v/v), o.n.	20%	(traces)
5	CrO_3 (0.5 equiv.) NMO (3 equiv.)	CH_2Cl_2, AcOH/H_2O (9:1 v/v), o.n.	40%	(traces)
6	NMO (3 equiv.)	CH_2Cl_2, o.n.	-	-

[a] Reaction performed on 100 mg of **3** (0.20 mmol). *Reagents and conditions*: **3** in the reported solvent, CrO_3 in AcOH/H_2O (9:1, v/v) and/or NMO, r.t. [b] Determined as **3** consumption by ^{1}H-NMR analysis of the crude reaction mixture. o.n.: overnight.

The best outcome (Table 5, entry 2) was then explored for solvent and phase-transfer effect using the set-up depicted in Figure 3. Initially, the reaction was performed in CH_2Cl_2 to avoid workup operations. However, at both high and low flow rates, in the presence of TEAI under ultrasound irradiation, a poor conversion was detected (Table 6, entries 1-3). This might be ascribable to the formation of H_2O/CrO_3 dots or CrO_3 precipitation. Accordingly, the monophasic mixture of

AcOH/H_2O (9:1, v/v) allowed to obtain the target product **4** in 75% yield (Table 6, entry 4). Under these conditions, reduction of CrO_3 equiv. (1.1) was negative for the reaction outcome (Table 6, entry 5).

Table 6. E-ring oxidative cleavage of pseudodiosgenin-3,26-diacetate (**3**) to diosone (**4**) under flow conditions: effect of solvents and phase transfer agents.[a]

Entry	CrO_3 equiv.	Flow rate (mL/min)	Phase transfer (Pump B)	Solvent B	Conversion yield[b]	Yield[b]
1[c]	1.4	2	-	CH_2Cl_2	-	(traces)
2[d]	1.4	0.6	TEAI (0.02 equiv.)	CH_2Cl_2	60%	30%
3[d,e]	1.4	0.6	TEAI (0.02 equiv.)	CH_2Cl_2	60%	30%
4	1.4	2	-	AcOH/H_2O (9:1, v/v)	Quantitative	75%
5	1.1	2	-	AcOH/H_2O (9:1, v/v)	70%	62%

[a] Reaction performed on 100 mg of **3** (0.20 mmol) using the flow set-up reported in Figure 3. *Reagents and conditions*: **3** 0.05 M in CH_2Cl_2, CrO_3 (1.4 equiv.) 0.07 M in AcOH/H_2O (9:1, v/v), phase transfer agent, r.t. [b] Determined as **3** consumption by ^{1}H-NMR analysis of the crude reaction mixture. [c] H_2O (and CrO_3) dots observed; H_2O necessary to completely solubilise CrO_3. [d] CrO_3 precipitation in loop and reactor. [e] The reaction was conducted irradiating the reactor with ultrasound.

The possibility to perform the final hydrolysis/elimination step by a telescoped approach was next evaluated. Indeed, taking advantage from the acidic environment deriving from the previous transformation, the crude reaction mixture from the CrO_3-mediated oxidative cleavage of **3** was readily flowed into a second 10 mL thermostated coil reactor spaced by a residence time reactor, instrumental to collect the reaction mixture before changing the flow rate for the hydrolytic step

(Figure 4). The outflowing crude **1** was detected by in-line UV sensor before being collected into a fraction collector.

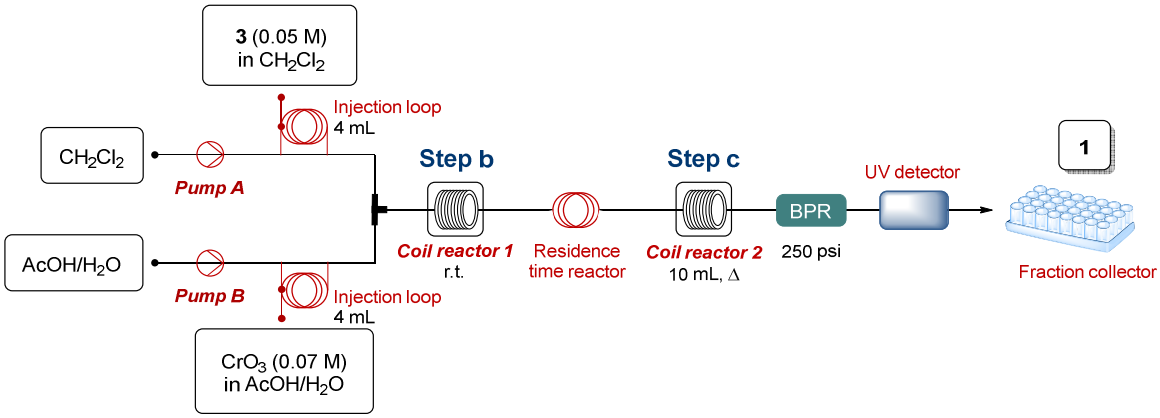


Figure 4. Flow set-up used for the optimization of the reaction conditions reported in Table 7.

Initially, both steps b and c were performed at 1 mL/min to give 40% conversion, 30% yield of **4** and traces of the target product **2** (Table 7, entry 1). When the solution of **3** was reacted with CrO_3 dissolved in AcOH/H_2O, at 2 mL/min flow rate for the oxidative step and 0.2 mL/min for the hydrolysis step, mixtures composed by starting material **3**, **4** and **1** were obtained (Table 7, entries 2 and 3). Having noted the necessity to pre-solubilize the oxidant in H_2O to form the active oxidizing species (Table 7, entries 4 and 5), a remarkable high yield (>90% from **3** to **1**) was obtained when the reaction was performed at 150 °C and using 10% of H_2O in line B.

Table 7. Study of 16-dehydropregnenolone acetate (**1**) synthesis from pseudodiosgenin-3,26-diacetate (**3**) under flow conditions.[a]

Entry	H_2O	Flow rate (mL/min)		Temperature Reactor 1	Temperature Reactor 2	Results[b]		
		Step b	Step c			3	4	1
1[c]	10%	1	1	5 mL	130 °C	60%	30%	(traces)
2[c,d]	10%	2	0.2	10 mL	130 °C	18%	30%	50%
3[c]	20%	2	0.2	10 mL	130 °C	12%	37%	51%
4[e]	20%	2	0.2	10 mL	130 °C	-	42%	58%
5[e]	10%	2	0.2	10 mL	130 °C	-	40%	60%
6[e]	10%	2	0.2	10 mL	145 °C	-	(traces)	90%
7[e]	10%	2	0.2	10 mL	150 °C	-	-	95%

[a] Reaction performed on 100 mg of **3** (0.20 mmol) using the flow set-up depicted in Figure 4. *Reagents and conditions*: **3** 0.05 M in CH_2Cl_2, CrO_3 (1.4 equiv.) 0.07 M in AcOH/H_2O (9:1 or 8:2, v/v), r.t., then 130-150 °C. [b] Relative percentages and yields were determined by ^{1}H-NMR analysis of the crude reaction mixture. [c] CrO_3 was dissolved in the mixture of AcOH and H_2O. [d] CrO_3 precipitation. [e] CrO_3 was dissolved in H_2O, then AcOH was added.

Diverse attempts aimed at reducing the total reaction time over the two steps were also performed by applying ultrasound or microwave irradiation at reactor 2 or by adding catalytic amounts of *p*TSA. Unfortunately, none of them gave better results (Table 8, entries 1-5). Finally, 1.1 equiv. of CrO_3 afforded 1 in 80% and 60% yield using conventional and microwave heating, respectively (Table 8, entries 6 and 7).

Table 8. Attempts to optimise 16-dehydropregnenolone acetate (**1**) synthesis from pseudodiosgenin-3,26-diacetate (**3**) in flow conditions.[a]

Entry	CrO_3 equiv.	Catalyst	Reactor 2[b]	Flow rate (mL/min)	Results[c]		
					3	**4**	**1**
1	1.4	-	Coil, ultrasound, 54-62 °C	0.2	-	75%	*(traces)*
2	1.4	*p*TSA (0.1 equiv.)	Coil, 150 °C	0.4		23%	68%
3	1.4	-	Microwave, 150 °C	0.4		30%	70%
4	1.4	-	Microwave, 160 °C[d]	0.4			-
5	1.4	-	Microwave, 150 °C	0.3		18%	66%
6	1.1	-	Coil, 150 °C	0.2		12%	80%
7	1.1	-	Microwave, 150 °C	0.2	10%	20%	60%

[a] Reaction performed on 100 mg of **3** (0.20 mmol). *Reagents and conditions*: **3** 0.05 M in CH_2Cl_2, CrO_3 (1.1 or 1.4 equiv.) 0.07 M in AcOH/H_2O (9:1, v/v), r.t., then heating. [b] 10 mL. [c] Relative percentages and yields were determined by ^{1}H-NMR analysis of the crude reaction mixture. [d] The set temperature was not reached.

The information gathered throughout these feasibility studies allow us to develop a more comprehensive process understanding and as a result enabled us to define the requirement and appropriate set-up for a flow processing method.

2.2 Telescoped flow synthesis of 16-dehydropregnenolone acetate from diosgenin

An integrated, telescoped flow transformation of diosgenin (**2**) into 16-DPA (**1**) was designed in order to reduce the downstream operations towards an easy scalable process. The first task to be tackled was the removal of acidic traces from the crude pseudodiosgenin-3,26-diacetate (**3**) to prevent pump injury caused by the acidic media and salt precipitation. To this end, we decided to attempt an in-line work-up (Figure 5). Initially, the reaction mixture was treated with a 0.2 M aqueous solution of NaOH and, after phase separation, the organic layer was pumped into the system. A pump plunger failure was observed as ascribed to salt precipitation. Nevertheless, the final product **1** was obtained in 65% yield along with traces of diosgenin (**2**). Therefore, an aqueous washing was performed and pseudodiosgenin-3,26-diacetate (**3**) was obtained in 75% yield after a simple extraction. The crude was further processed to provide 16-DPA (**1**) in 60% yield without any pump failure. Finally, after reacting the mixture of **2** and $BF_3{\cdot}Et_2O$ with ATFAA at room temperature, a workup with a 0.5 M aqueous solution of $NaHCO_3$ was performed to obtain **3** in 80% yield.

The improved flow setup was applied to the telescoped synthesis of 16-DPA (**1**) from diosgenin (**2**) (Figure 5). Therefore, a solution of diosgenin (**2**) and $BF_3{\cdot}Et_2O$ (2.0 equiv.) in dry CH_2Cl_2 was mixed with freshly prepared ATFAA (11.0 equiv.) in a T-junction and pumped through the coil reactor maintained at room temperature with a total flow rate of 1.0 mL/min. The stream was then combined in a T-shaped mixing element with a 0.5 M aqueous solution of $NaHCO_3$, and the biphasic system was sent to a membrane separator. The organic layer was filtered on a Nylon syringe filter after the aqueous washing, and used as a stock solution of **3** for the next step. Therefore, the solution of pseudodiosgenin-3,26-diacetate (**3**) was mixed with CrO_3 in $AcOH/H_2O$ (9:1, v/v) in a T-junction and the solution was pumped in a 10 mL coil reactor at room temperaure

with at 2 mL/min flow rate. The reaction mixture was collected into a residence time reactor before entering a 10 mL coil reactor heated at 150 °C. After a residence time of 50 min, the outflow was combined with a 0.5 M aqueous solution of $NaHCO_3$ and CH_2Cl_2 (0.5 mL/min for each pump) and the organic layer was separated by a gravity extraction funnel. Concentration of the organic phase and automated flash chromatography of the crude mixture led to the target product **1** in 95% yield over two steps (overall yield: 75%).

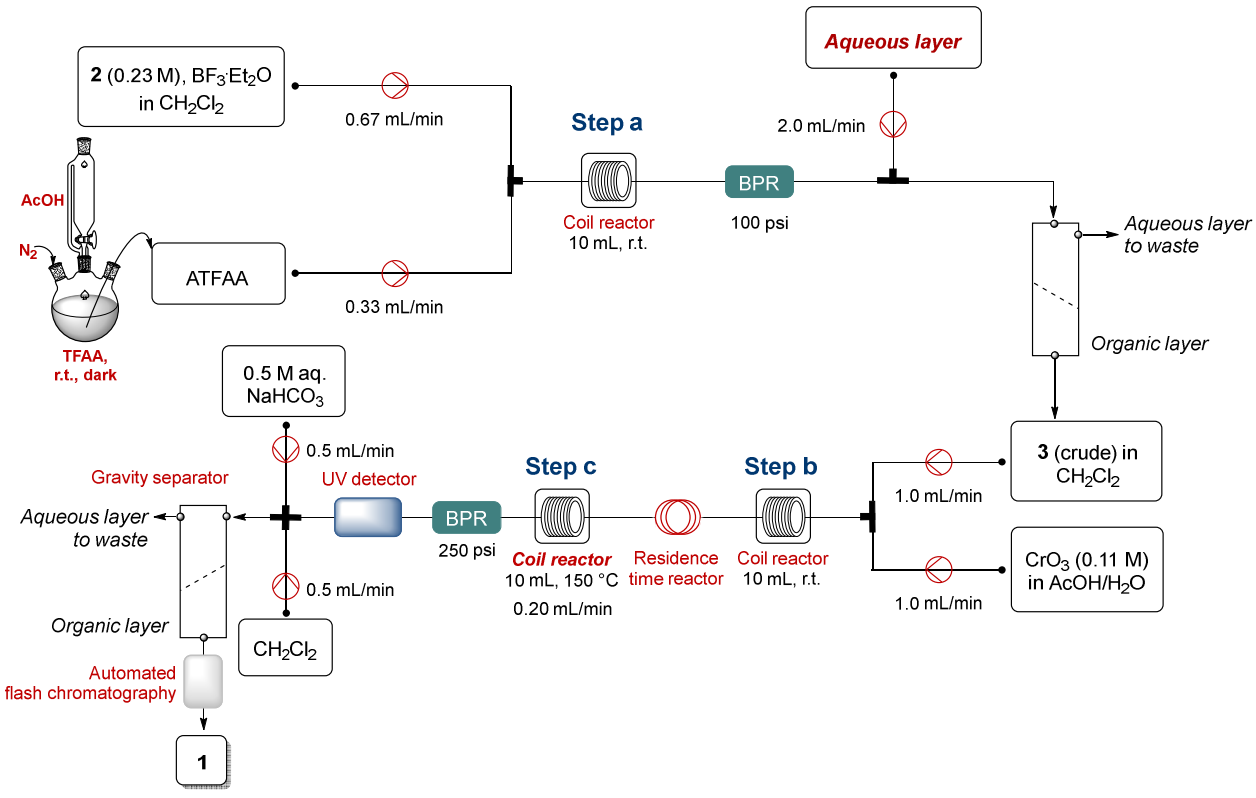


Figure 5. Integrated flow set-up for the investigation of diosgenin (**2**) degradation to 16-dehydropregnenolone acetate (**1**).

Conclusions

In summary, the set-up and assessment of the telescoped, continuous flow synthesis of 16-DPA (**1**) from diosgenin (**2**) was accomplished. Besides the known benefits offered by flow processing over batch synthesis, our method supersedes bottlenecks like the use of harsh reaction conditions and showed important advantages and improvements with respect to previous syntheses (Table S4, Supporting Information). These include higher efficiency and yields under milder reaction conditions, the use of lower amount of reagents, reduced manual handling, simple downstream procedures, that resulted in high quality material and safety standards. Moreover, the process exhibited a productivity of 2.1 g/day (for single reactor) and it can be easily monitored by in-line analysis and assisted by automation software. Preliminary attempts of increasing productivity (the first step can generate up to 150 g/d of intermediate **3**, see Supporting Information) using higher concentrations, peristaltic pumps, and larger tubing reactors showed that the method is robust and amenable for future process intensification works and kilo scale preparations. In this frame, future efforts directed towards the optimization of the oxidative cleavage can be foreseen. Our results suggest that the implementation of an OsO_4-catalized continuous method integrated with an appropriate reagent loading system and in-line scavenging as thiurea resins would improve the overall synthetic process limiting toxicity, handling, and disposal issue while enabling catalyst recovery and reuse.

Supporting Information. Experimental procedures, images of flow equipment used, spectroscopic characterization and copies of NMR, FT-IR, EICs and HRMS spectra, and HPLC HPLC chromatograms are provided. The following files are

Corresponding Author

*Prof. Antimo Gioiello, e-mail: antimo.gioiello@unipg.it

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ABBREVIATIONS

16-DPA, 16-dehydropregnenolone acetate; Ac_2O, acetic anhydride, ATFAA, acetic trifluoroacetic anhydride; DBU, 1,8-diazabicyclo(5.4.0)undec-7-ene; MEK, methyl ethyl ketone; NMO, *N*-methylmorpholine-*N*-oxide; *p*TSA, *p*-toluenesulfonic acid; TEAI, tetraethylammonium iodide; TEBA, triethyl benzyl ammonium bromide; TFAA, trifluoroacetic anhydride.

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