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Article

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Hemisynthesis of 2,3,4-^{13}C3-1,4-androstadien-3,17-dione: A key precursor for the synthesis of ^{13}C3-androstanes and ^{13}C3-estranes.

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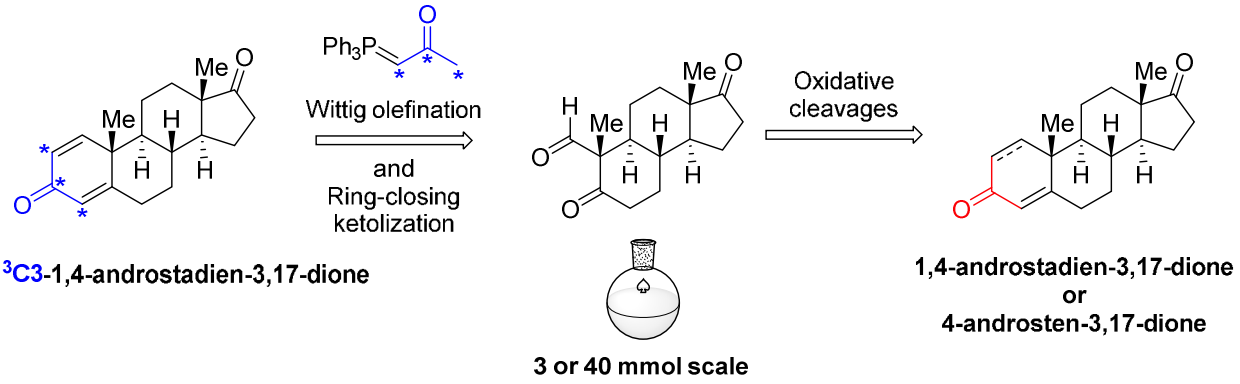


Abstract

In this contribution, we describe two simple and efficient routes for the preparation of keto-aldehyde **1**, a key intermediate for the synthesis of ^{13}C3-androstanes and ^{13}C3-estranes. In the first route, the targeted aldehyde **1** was obtained in 40% overall yield from 1,4-androstadien-3,17-dione (3 mmol scale) via a two-step sequence involving a one-pot, abnormal ozonolysis/sulfur oxidation/retro-Michael/ozonolysis process. Alternatively, a second route from 4-androsten-3,17-dione, using a six-step sequence, was optimized to produce 40 mmol batches of the key intermediate **1** in 42% overall yield. At the final stage, the A-ring was reconstructed through a Wittig reaction with the 1-triphenylphosphoranylidene-^{13}C3-2-propanone **2**, followed by an intramolecular condensation assisted by thioacetic acid *via* a Michael addition/retro-Michael reaction sequence to provide 2,3,4-^{13}C3-1,4-androstadien-3,17-dione.

Key words: *Steroids, ^{13}C-labeled compounds, hemisynthesis, oxidation, ozonolysis, Wittig reaction, Palladium-catalyzed decarbonylative dehydration*.

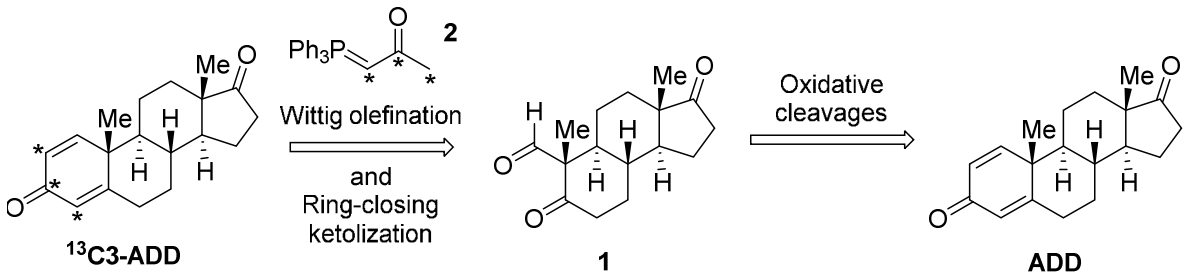
Introduction

Due to the wide spectrum of biological activitites of both natural and synthetic steroids,[1] the detection and quantification of steroidal residues in various biological materials are crucial for clinical and pharmacological studies. For instance, it is extremely useful for absorption, distribution, metabolism, and excretion (ADME) studies leading to the development of novel steroidal drugs[2] and for doping prevention to fight against the use of anabolic steroids[3] in sports competitions. In addition, the quantification of steroid traces is an issue for environmental protection. For example, it has been demonstrated that estrogen contained in birth control pills, and thus eliminated by the body *via* the urinary system and discharged into wastewaters, is an endocrine disruptor of aquatic fauna.[4]

The analytical technique of stable isotopic dilution[5] (SID) by Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry (LC-MS) is widely employed today to conduct accurate and reproducible steroid quantification in clinical and biochemical studies. This first-choice technique requires the use of an internal standard, an isotopologue labeled with stable isotopes (usually ^{13}C or deuterium) with a high isotopic purity and presenting a difference of at least three mass units with the parent unlabeled compound. This second criterion nullifies the effect of the natural abundance of heavy isotopes contained in the labeled standard. Deuterium-labeled standards can be readily accessed by post-synthetic hydrogen-deuterium exchange reactions catalyzed by transition metals (mainly palladium and platinum), using deuterium oxide (D_2O) as the cheapest deuterium source. Compared to conventional synthetic approaches, post-synthetic hydrogen-deuterium exchange is an easy and economical methodology to prepare multi-deuterated compounds. Since the pioneering work of Sajiki,[6] many deuterated compounds have been prepared by hydrogen-deuterium exchange catalyzed by transition metals, with D_2O as a deuterium source, under a hydrogen atmosphere.[7] To avoid the handling of gaseous hydrogen, Derdau and Atzrodt[8] significantly improved this methodology by using deuteride donors, such as $NaBD_4$, for pre-activation of the catalyst. Nevertheless, the disadvantages of hydrogen-deuterium exchange include non-specific and incomplete deuteration affording deuterated standards with an inevitably uneven isotope distribution.

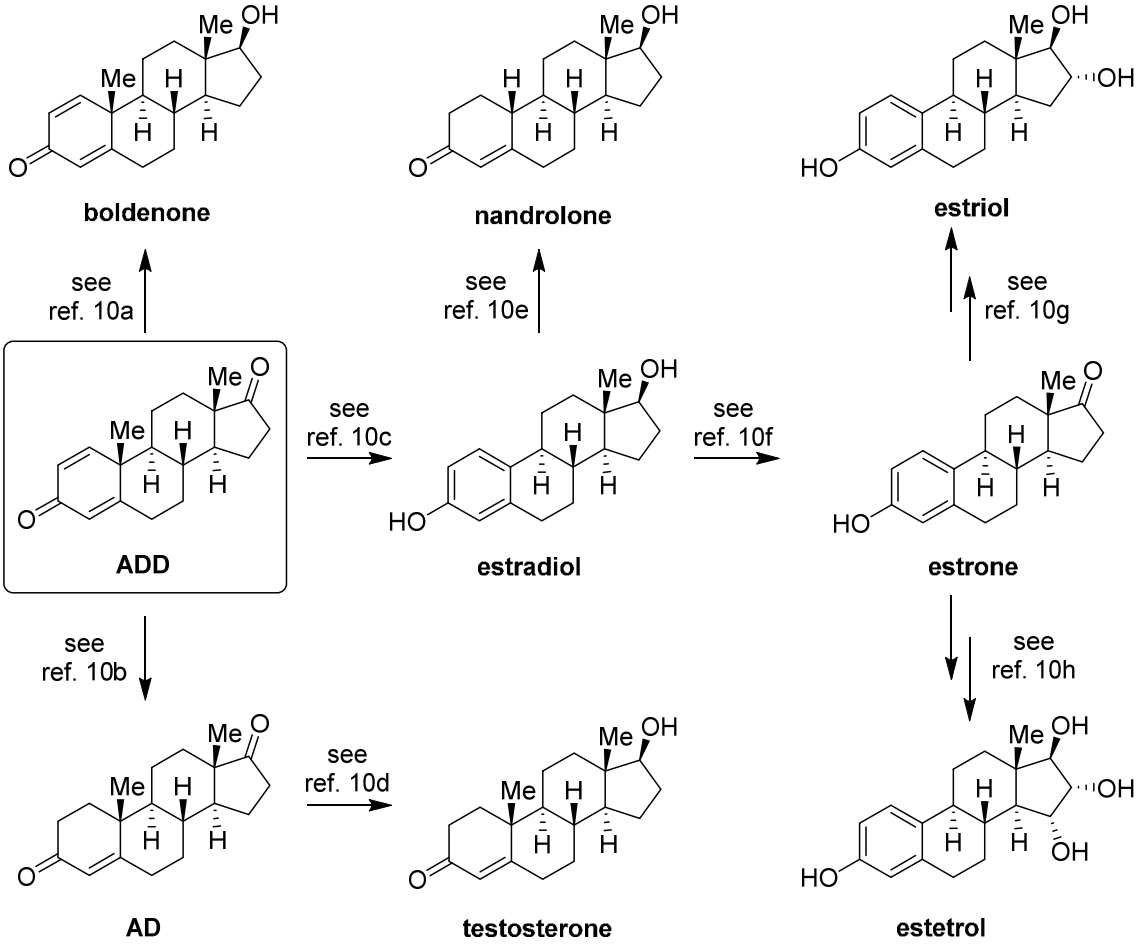
Moreover, it has been frequently pointed out that the use of deuterated standards suffers from deuterium-hydrogen back exchange at different steps of the analysis. For these reasons, ^{13}C-labeled standards are usually preferred to deuterium-labeled standards, due to the negligible isotopic effect during ^{13}C-labeling and the robustness of the standards during metabolic processes and/or pre-analytical chemical treatments of the samples, and despite the cost of the ^{13}C-labeled starting materials and/or reagents used for their syntheses.

To address the key issue of the synthesis of ^{13}C-labeled steroids at reasonable cost and, ideally, with high versatility, we describe here an efficient and short hemisynthesis of 2,3,4-^{13}C3-1,4-androstadien-3,17-dione (**2,3,4-^{13}C3-ADD**) starting from commercially available unlabeled **ADD** and using the triply labeled ^{13}C [1,2,3-^{13}C3]-1-(triphenylphosphoranylidene)-2-propanone **2** as the three-carbon building block (see Scheme 1). The latter was efficiently prepared in three steps, using well-established procedures, from two relatively inexpensive reagents: [^{13}C2]-acetyl chloride and ^{13}C-iodomethane. From a strategic point of view, it was crucial to use this precious ^{13}C3-labeled reagent at the final stage of our synthesis to incorporate the three ^{13}C atoms into the framework of **ADD**.



Scheme 1: Retrosynthesis for **$^{13}C_3$-ADD**.

It should be pointed out that the choice of **ADD** as target was dictated by the fact that the resulting **^{13}C3-ADD** could be converted into other important members of this steroid family, such as ^{13}C3-androgens and ^{13}C3-estrogens, using a limited number of well-established transformations (see Scheme 2).[9]



Scheme 2: Preparation of various [2,3,4-^{13}C3]-enriched steroids from [2,3,4-^{13}C3]-**ADD**.[10]

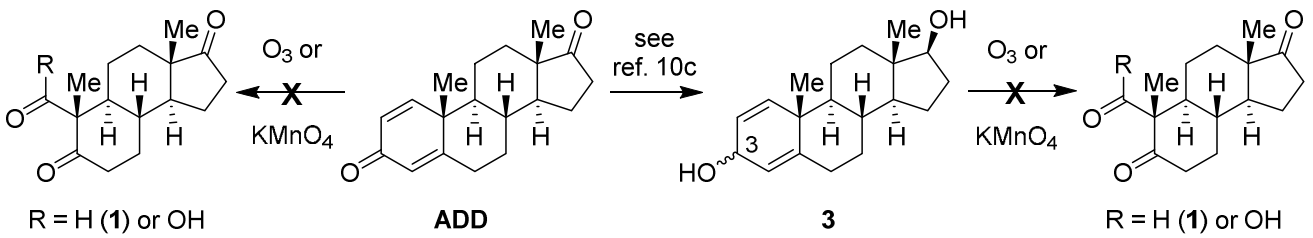
Two synthetic routes to [3,4-^{13}C2]-enriched[11] and [2,3,4-^{13}C3]-enriched[12] steroids have been previously reported. In the first one, the enone of the A-ring of **ADD** was opened either by ozonolysis[11a] followed by treatment with hydrogen peroxide or by oxidative cleavage with $KMnO_4/KIO_4$.[11b] The treatment of the resulting keto acid with Ac_2O or AcCl in the presence of catalytic amounts of $HClO_4$ led to the enol lactone intermediate, which was converted into the corresponding lithium enolate, then subjected to acylation with [1,2-^{13}C2]-acetyl chloride. Acidic treatment led to hydrolysis of the lactone and the resulting β-keto acid underwent spontaneous decarboxylation, affording the dione. Subsequent Robinson annulation gave the [3,4-^{13}C2]-enriched steroid. This efficient and straightforward four-step sequence allowed the incorporation of only two ^{13}C atoms into the steroid framework. In the second route, Vierhapper[12] described the synthesis of [2,3,4-^{13}C3]-enriched testosterone from unlabeled testosterone *via* a key 10-formyl-5-oxo-des-A intermediate similar to compound **1** depicted in Scheme 1. The first step of the sequence involved a *tert*-

butoxide/18-Crown-6 mediated autoxidation of the kinetic enolate of testosterone under a stream of molecular oxygen over a long period of time (up to 90 hours). The resulting lactone intermediate was then reduced in a second step into the corresponding 2-oxasteroid. The targeted 10-formyl-5-oxo-des-A intermediate was obtained following silylation of the 17β-hydroxyl, and ozonolysis and oxidation of the primary alcohol into the corresponding aldehyde.

Results and discussion

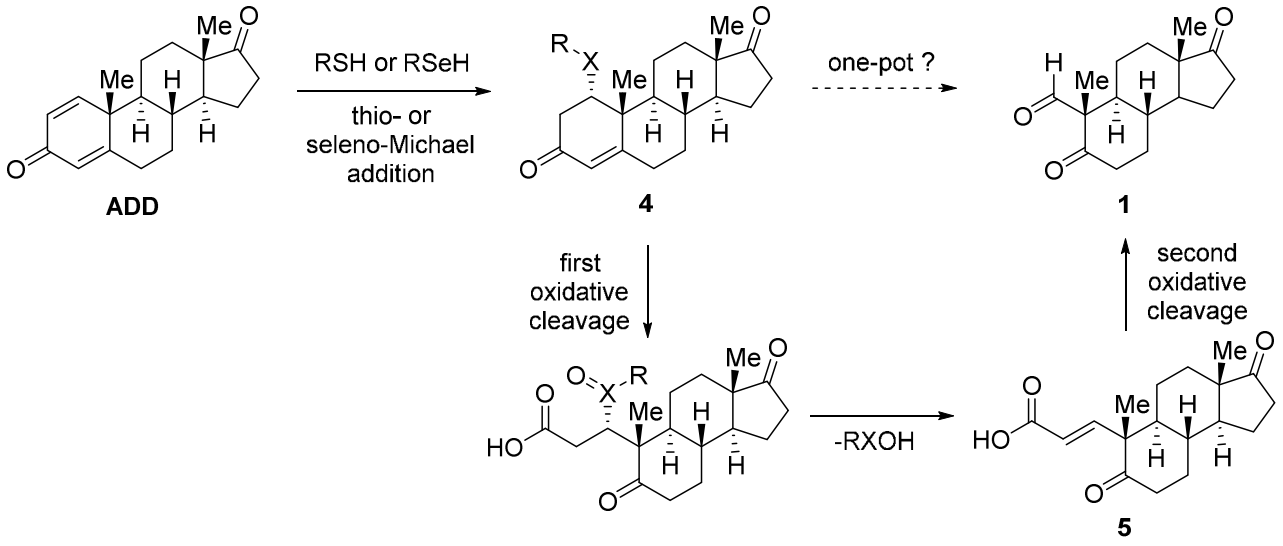
In the light of these previous works, it was clear to us that the 10-formyl-5-oxo-des-A intermediate was the best common intermediate to prepare a large variety of [2,3,4-^{13}C3]-enriched steroids. Following Vierhapper's work,[12] we became interested in both shortening the synthetic route and finding an alternative to the tricky *tert*-butoxide/18-Crown-6 mediated autoxidation used in the first step of the sequence to provide a safe and reliable route to large quantities of 10-formyl-5-oxo-des-A intermediate **1**.

To this end, we initially focused our attention on the double oxidative cleavage of the two carbon-carbon double bonds in the A-ring of **ADD**. This strategy would rapidly deliver the desired keto-aldehyde **1** (Scheme 3). Unfortunately, the A-ring of **ADD** was found to be completely inert under standard ozonolysis conditions and under oxidative cleavage conditions with potassium permanganate. Breaking the electronic conjugation of the A-ring by a regioselective reduction of carbonyl at C-3, with concomitant reduction of the carbonyl at C-17, has been considered.[10c] The resulting cyclohexadienol **3** was found to be rather unstable and led to either aromatization of the A-ring by a benzene-dienol rearrangement[13] or a complex mixture of degradation products under oxidative cleavage conditions.



Scheme 3: Initial attempts at the double oxidative cleavage of the A-ring of **ADD** and cyclohexadienol **3**.

Turning to this critical double simultaneous oxidative cleavage of the A-ring carbon-carbon double bonds of **ADD**, we envisaged that the cleavage could be achieved in a sequential manner using a one-pot procedure depicted in Scheme 4. A regioselective thio- or seleno-Michael addition onto the more reactive carbon-carbon double bond of **ADD** was considered as a temporary protection. This would allow the oxidative cleavage of the remaining 4,5-carbon-carbon double bond by ozone to be achieved. Under these oxidative conditions, we expected to form *in situ* the corresponding sulfoxide (or selenoxide), which could regenerate spontaneously the masked carbon-carbon double bond *via* a β-elimination reaction. The resulting α,β-unsaturated acid **5** was expected to undergo oxidative cleavage under the atmosphere of ozone and thus provide the desired 10-formyl-5-oxo-des-A intermediate **1** following this one-pot multi-step process.

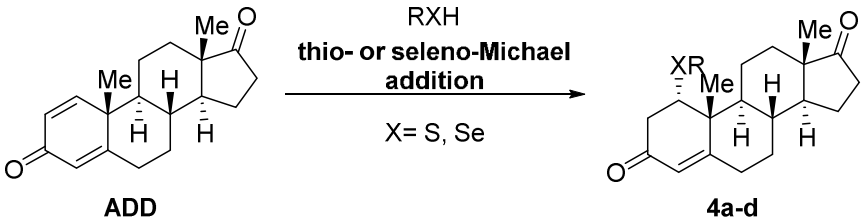


Scheme 4: Plausible synthetic route to keto-aldehyde **1** involving a one-pot multi-step sequence from **4a–d**.

Four hetero-Michael adducts **4a–d** of **ADD** were prepared to evaluate our strategy. Following known procedures,[14] thioacetate **4a** and the phenyl sulfide **4b** adducts were obtained in high yields from **ADD** (see Table 1). In the case of compound **4b**, slightly modified reaction conditions were employed to avoid the use of thiophenol as solvent and metallic sodium. Under the same conditions, the ethanethiol adduct **4c** was obtained with a moderate yield of 43%. To our delight, the seleno-Michael addition promoted by a catalytic amount of γ-cyclodextrin gave the seleno-adduct **4d** in 92% yield under smooth reaction conditions. It is worth noting that the use of β-cyclodextrin did not lead to a complete conversion of **ADD**.[15] In all cases,

only the diastereoisomer resulting from the nucleophilic attack on the less hindered α-side was obtained. This preference for the attack on the α-side was confirmed by an X-ray diffraction crystallography analysis of the thiophenol adduct **4b** (see Supporting Information).

Table 1: Preparation of adducts **4a-d** from **ADD**.



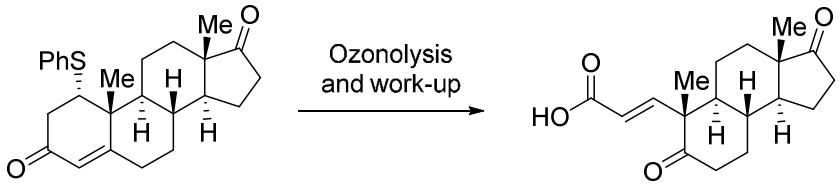
Entry	Conditions	Yield [a]
1	AcSH (10.3 equiv), THF/MeOH/H_2O (10:10:1), rt, 12 h	**4a**: 89 %
2	PhSH (10 equiv), PhSNa (5 equiv), THF, rt, 48 h	**4b**: 83 %
3	EtSH (4 equiv), NaH (1 equiv), THF, rt, 96 h	**4c**: 43 %
4	PhSeH (1 equiv), γ-CD (10 mol%), H_2O/Acetone (15:2), rt, 6 h	**4d**: 92 %

[a] Isolated yield.

With these four hetero-Michael adducts **4a-d** in hand, we investigated the abnormal ozonolysis[16,17] of the remaining enone function (see Table 2). We started our investigations with thiophenol adduct **4b** as the model substrate. When a short contact (3 min) with ozone was applied in dichloromethane at –78 °C, followed by purging the system with Argon to remove the excess of ozone, then warming to room temperature, only a mixture of the starting material **4b** and **ADD** was obtained, with no traces of the desired keto-aldehyde **1** or alternatively, keto-acid intermediate **5**, being detectable in the crude reaction mixture (Table 2, Entry 1). This result clearly indicated that the first transformation occurring in this process is the oxidation of the sulfide into the corresponding sulfoxide (or sulfone), which undergoes a retro-Michael reaction to afford **ADD**. At this stage it remained unclear whether the β-elimination occurred at low temperature or, as expected, during the warm up to room temperature. Keto-acid **5** was observed as the minor product in the reaction mixture (28%) when ozone bubbling was applied for a longer period of time (30 min) at –78 ºC, with the major component still being **ADD** (Table 2, Entry 2). As **ADD** was found to be

unreactive under ozonolysis conditions, the formation of α,β-unsaturated acid **5** (in its *E*-configuration) in this second experiment tends to indicate that the β-elimination of the sulfoxide moiety occurred upon warming to room temperature, after ozone has been removed from the system, and not at low temperature. The use of methanol and acetic acid as co-solvents did not improve the conversion (Table 2, Entries 3 and 4). Interestingly, the use of ethyl acetate as a solvent for the ozonolysis considerably improved the conversion (Table 2, Entries 5–7) and, after one hour of reaction at –78 ºC then warming to room temperature, only the expected keto-acid **5** was observed in the crude mixture (Table 2, Entry 7).

Table 2: Study of the reaction conditions for the ozonolysis of **4b**.



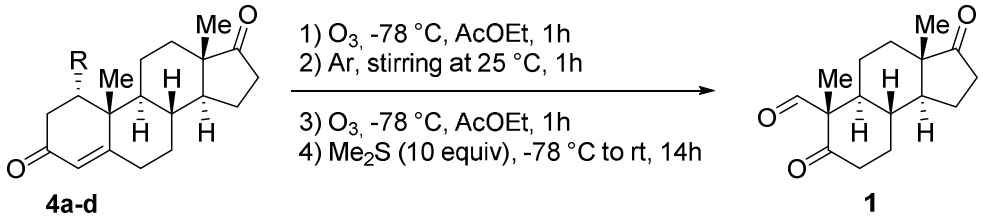
4b **5**

Entry[a]	Conditions	Treatment	Products (%)[b]
1	O_3, DCM, 3 min[c]	Me_2S (10 equiv)	**ADD** (55): **4b** (45)
2	O_3, DCM, 30 min	Me_2S (10 equiv)	**ADD** (72): **5** (28)
3	O_3, DCM, MeOH, 45 min	NaOH aq.	**ADD** (80): **5** (traces)[d]
4	O_3, DCM, MeOH, AcOH, 1 h 30 min	NaOH aq.	**ADD** (60): **5** (traces)[d]
5	O_3, AcOEt, 45 min	Me_2S (10 equiv)	**ADD** (18): **5** (82) [c]
6	O_3, AcOEt, 45 min	NaOH aq.	**ADD** (15): **5** (85)[d]
7	O_3, AcOEt, 1 h	Me_2S (10 equiv)	**ADD (–): 5** (>95)

[a] All reactions were performed on a 3 mmol scale, first at –78 °C under an atmosphere of O_3 for the period of time indicated, then at room temperature under an atmosphere of Argon; [b] Ratios were determined by ^{1}H NMR of the crude mixture; [c] The deep blue coloration, characteristic of a saturated ozone medium, was reached after 3 min of ozone bubbling; [d] Several unidentified degradation products were formed.

Having secured the abnormal ozonolysis/sulfur oxidation/retro-Michael sequence to form the α,β-unsaturated acid **5** from **4b**, we next evaluated the one-pot process in which the first ozonolysis intermediate **5** would be directly involved in a second ozonolysis reaction to provide the key keto-aldehyde **1**. The double-ozonolysis sequence was carried out on the four hetero-Michael adducts **4a–d**. For the first part of this one-pot, multi-step protocol, we used the optimized conditions described in Table 2 to reach intermediate **5**. The reaction mixture was then cooled down back to –78 °C, treated with ozone for one hour and finally reacted with an excess of dimethyl sulfide. As presented in Table 3, the phenylsulfide **4b** gave the best yield (43%) (Table 3, Entry 2), while other thio- or seleno-Michael adducts **4a** and **4c–d** were found to be less efficient (Table 3, Entries 1, 3 and 4). To explain the formation of the keto-aldehyde **1**, we proposed the mechanism depicted in the supporting information.[18]

Table 3: One-pot process for the synthesis of **1** from **4a-d**.



4a-d **1**

Entry [a]	*Substrate*	*R*	*Yield (%)* [b]
1	**4a**	AcS	0
2	**4b**	PhS	43
3	**4c**	EtS	11
4	**4d**	PhSe	24

[a] All reactions were performed on 3 mmol of adduct **4**; [b] Isolated yield.

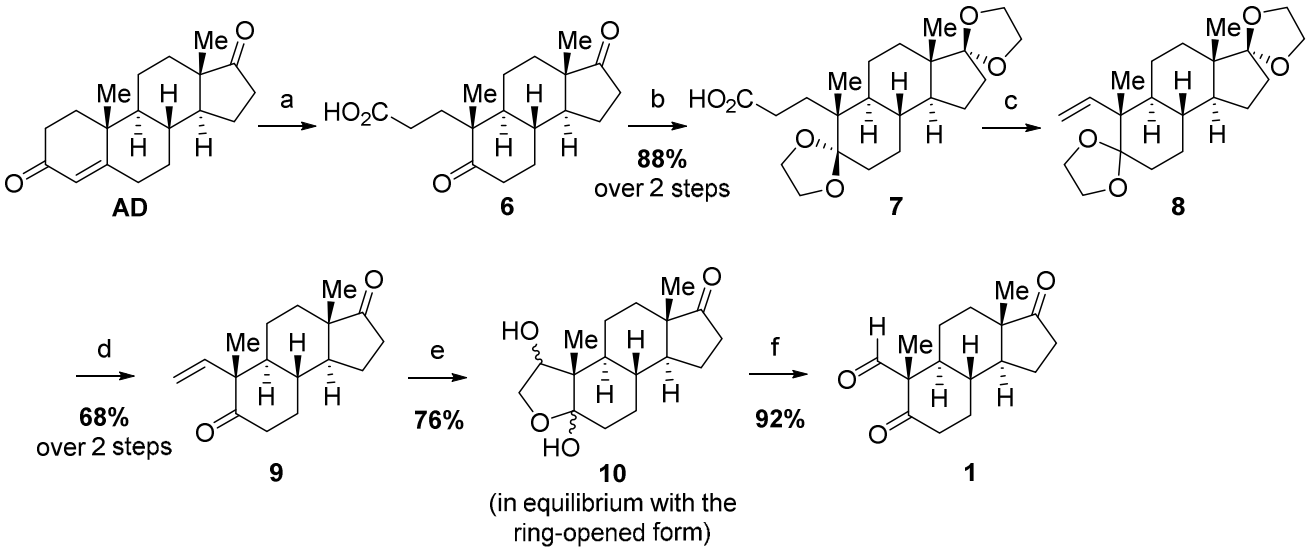
To the best of our knowledge, this approach represents the shortest access reported so far to the keto-aldehyde **1**, a key precursor of ^{13}C3-labeled steroids, from a commercially available steroid. Overall, this one-pot, multi-step sequence proceeded in an acceptable 36% yield on a 3 mmol scale from **ADD**, *via* the thiophenyl sulfide **4b** intermediate. This sequence provided ca. 500 mg of pure keto-aldehyde **1**, which in

the field of the synthesis of labeled compounds is a reasonable scale. Unfortunately, on larger scales the overall yield dropped to 20–25%.

Given the difficulties encountered in carrying out the direct double oxidative cleavage of the cyclic dienone function of **ADD** (or its dienol derivative), we examined an alternative route to produce keto-aldehyde **1** from commercial 4-androsten-3,17-dione (**AD**), without the need for any ozonolysis steps. We focused our attention on the use of the diketo-acid **6**, which is easily accessible in a single step by an oxidative cleavage of the 4-en-3-one function of **AD**[19] (Scheme 5). In their previous works, Vierhapper and co-workers attempted to convert carboxylic intermediates such as compound **6** into the corresponding vinyl derivatives *via* an oxidative decarboxylation reaction using either lead tetraacetate or diacetoxy-iodobenzene (DAIB).[12b] Despite their efforts to optimize these oxidative decarboxylation reactions, the best yields with lead tetraacetate and DAIB remained modest (42 and 34%, respectively) and the targeted terminal olefin was obtained only in small amounts.

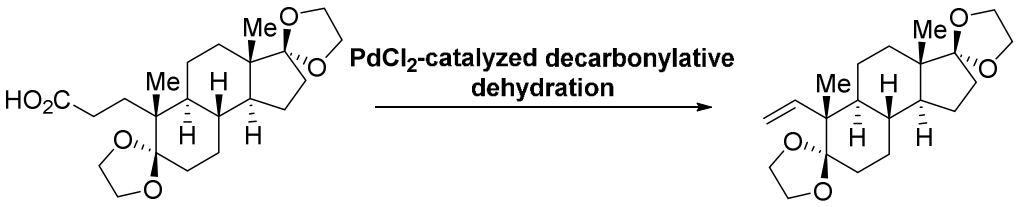
Inspired by these seminal works, we evaluated the palladium-catalyzed decarbonylative dehydration of the carboxylic acid **7** as a key step to furnish the key vinyl **8**. The latter could then lead to keto-aldehyde **1** after a second oxidative cleavage. Although this reaction has been known for a long time and used for the production of olefins from biomass feedstocks,[20] surprisingly we found very few examples of metal-catalyzed decarbonylative dehydration applied in a multi-step synthesis.[21] First, the palladium-catalyzed decarbonylative dehydration conditions published by Scott[22] on aliphatic carboxylic acids were evaluated on our substrate **7**. It should be pointed out that both ketone functions at C-3 and C-17 on compound **6** were protected to avoid the formation of the cylic enol-lactone intermediate[11a] during the activation of the carboxylic acid with Ac_2O. Starting material **7** remained essentially unchanged even after prolonged reaction times (up to three days) and only low yields of alkene **8** were obtained (Table 4, Entries 1–4). The quaternary center in acid **7** could be responsible for the lack of reactivity compared to the results reported in the literature with linear aliphatic carboxylic acids. Gratifyingly, after some experimentation, we noted that higher reaction temperatures (up to 245 °C) provided a good conversion. In particular, we found that the combination of catalytic amounts of palladium dichloride and triphenylphosphine at 250 °C previously described by Miller[23] was effective in furnishing the expected olefin **8** in good yields (Table 4, Entries 5–7). Interestingly, decarbonylation of the acyl palladium intermediate was initiated when the reaction

temperature reached 245 °C, as noted by the appearance of an intense red coloration concomitantly with the liberation of CO from the reaction flask.



Scheme 5: Synthesis of **1** from **AD**. *Reaction conditions*: a) $KMnO_4$ (6 mol%), $NaIO_4$ (5.7 equiv), Na_2CO_3 (1.2 equiv), *i*-PrOH/H_2O (1:1), 70 °C, 4 h; b) Ethylene glycol (3 equiv), $(EtO)_3CH$ (3 equiv), PTSA.H_2O (10 mol%), DCM, reflux, 16 h; c) Ac_2O (1.1 equiv), $PdCl_2$ (3 mol%), PPh_3 (0.75 equiv), DMPU, 250 °C, 75 min ; d) AcOH/H_2O (1:1), reflux, 1 h; e) OsO_4 (5 mol%), NMO (4 equiv), 2,6-lutidine (2 equiv), Acetone/H_2O (2:1), rt, 60 h; f) SiO_2-supported $NaIO_4$ (1.7 equiv), DCM, rt, 4 h.

Table 4



Entry[a]	Additives	Ligand	Solvent	T °C, Time	Yield (%)[b]
1	Ac_2O (1 equiv), TEA (1 equiv)	DPE-Phos	DMPU	110 °C, 14 h	16
2	Ac_2O (1 equiv), TEA (1 equiv)	DPE-Phos	DMPU	115 °C, 18 h	19
3	Ac_2O (1 equiv), TEA (1 equiv)	DPE-Phos	DMPU	120 °C, 3 d	20
4	Ac_2O (1 equiv), TEA (1 equiv)	DPE-Phos	CH_3CN	82 °C, 18 h	18
5	Ac_2O (1 equiv)	PPh_3	DMPU	250 °C, 30 min	50
6	Ac_2O (1 equiv)	PPh_3	DMPU	250 °C, 75 min	62
7	Ac_2O (1.1 equiv)	PPh_3	DMPU	250 °C, 75 min	73

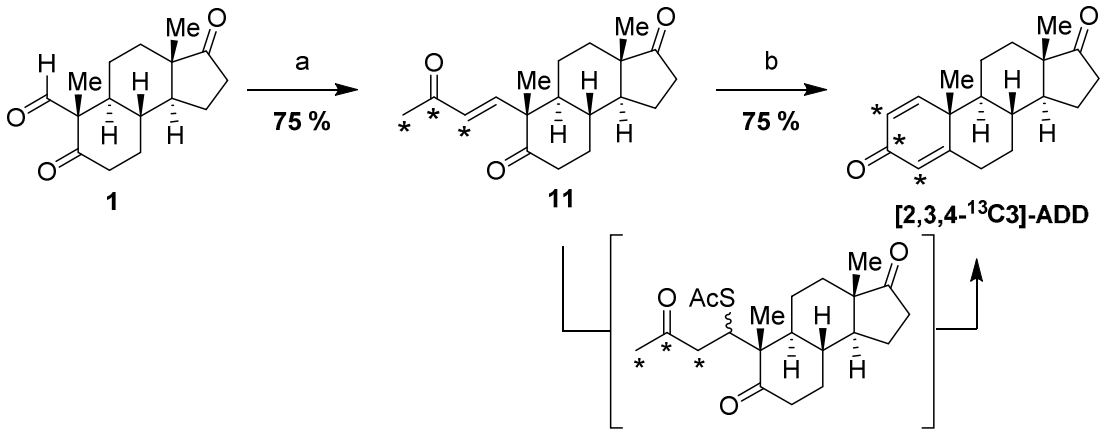
[a] All reactions were performed on 1 mmol of **7** with 3 mol% of $PdCl_2$; [b] Isolated yield.

This palladium-catalyzed decarbonylative dehydration reaction was routinely carried out on a 35 mmol scale to provide the desired vinyl intermediate **8**. The latter was then directly treated in refluxing aqueous acetic acid to afford olefin **9** in 68% yield over the two steps, after purification by flash chromatography on silica gel. Oxidative cleavage of **9** was successfully accomplished in two steps by Upjohn dihydroxylation, followed by treatment using silica gel-supported sodium metaperiodate,[24] to give the key keto-aldehyde **1** in 75% overall yield. It should be pointed out that dihydroxylation of the vinyl compound **9** afforded the expected diastereomeric diols, which were present in solution mostly in their corresponding hemiketal forms **10**.

Having secured two efficient routes to the key keto-aldehyde **1** following either a two-step sequence from **ADD** on a small scale (ca. 3 mmol) or a robust, ozone-free, six-step sequence from **AD** on a larger scale (ca. 40 mmol), delivering **1** in 36% and 42% overall yields, respectively, the last phase of our investigation was

the reconstruction of the A-ring to provide the targeted **^{13}C3-ADD**. To this end, the preparation of 1-triphenylphosphoranylidene-^{13}C3-2-propanone **2**, necessary for the incorporation of three ^{13}C atoms *via* a Wittig olefination, was accomplished in two steps,[25] starting from inexpensive ^{13}C-iodomethane and ^{13}C2-acetyl chloride. In order to address the issue of the cost of the synthesis, an improved sequence involving a ^{13}C2-acetyl imidazole intermediate was used (see Supporting Information for details).

The Wittig olefination of keto-aldehyde **1** with 1-triphenylphosphoranylidene-^{13}C3-2-propanone **2** was carried out using reported reaction conditions, furnishing exclusively the corresponding *E*-enone **11** in 75 % yield (Scheme 6). Extensive experimentation to increase the yield of this transformation under microwave activation,[26] or by using additives such as benzoic acid [27] or silica gel,[28] or by replacing the organic solvent by water,[29] proved unsuccessful. On the contrary, a negative effect was observed in most cases, as these conditions tended to favor the simple deformylation reaction of keto-aldehyde **1**.[30]



Scheme 6: Synthesis of ^{13}C3-**ADD** from keto-aldehyde **1**. *Reaction conditions*: a) **2** (1 equiv), *p*-xylene, 150 °C, 7 days; b) AcSH (1.2 equiv), PTSA.H_2O (0.3 equiv), toluene, 75 °C, 18 h, then DBU (2 equiv), rt, 30 min.

The last challenge of this synthesis was to develop a ring-closing ketolization of the (*E*)-enone **11** under reaction conditions enabling the isomerization of the (*E*)-carbon-carbon double bond into the (*Z*)-isomer in order to furnish the targeted **^{13}C3-ADD** in a single step. To the best of our knowledge, only one report describes the ketolization of an (*E*)-enone leading to the formation of a cyclic dienone under a refluxing

methanolic solution of potassium hydroxide.[31] Not surprisingly, these harsh conditions applied to (*E*)-enone **11** led to complete degradation. The geometry issue was addressed by treating (*E*)-enone **11** with a slight excess of thioacetic acid under acidic conditions. Under these conditions, the thio-Michael adduct underwent cyclization and the resulting intermediate was then treated with an excess of DBU to promote the retro thio-Michael reaction. Following this one-pot, three-step process, the targeted **^{13}C3-ADD** was obtained in good yield (75 %).

Conclusion

With current analytical techniques, appropriately ^{13}C-labeled steroids with a high isotopic purity and at least three ^{13}C-labels are crucial as internal standards for the analysis and quantification of steroids present in trace amounts in various matrices or as probes for clinical or biological investigations. Although there are several syntheses of ^{13}C-labeled steroids, their preparation requires a significant number of steps and supplies the quantities needed for routine use with difficulty. In this context, we have optimized a sequence to prepare ^{13}C-labeled steroids based upon keto-aldehyde **1**, which is routinely obtained by degradation of the A-ring of commercially available **ADD** or **AD**. Thus, we have developed two different routes; on a 3 mmol scale, a two-step sequence afforded this key compound **1** in 36% overall yield from **ADD**, whereas a more convenient large-scale synthesis (ca. 40 mmol) provided compound **1** following a six-step sequence in 42% overall yield from **AD**. Both strategies represent a significant improvement in existing methods in terms of efficiency, scale and cost. As an illustration of our investigations in this field, ^{13}C3-**ADD** was prepared from keto-aldehyde **1** by reconstruction of the A-ring *via* a Wittig reaction with the 1-triphenylphosphoranylidene-^{13}C3-2-propanone **2** followed by the intramolecular aldolization-crotonization condensation assisted by thioacetic acid.

Experimental Section

General information:

Water-sensible reactions were performed under an argon atmosphere in flame-dried glassware. All solvents used were reagent grade. Solvents for water-sensible reactions were purchased in anhydrous grade and were

used without further purification. ^{13}C-iodomethane (isotopic purity, 99 atom % ^{13}C) and ^{13}C2-acetyl chloride (isotopic purity, 99 atom % ^{13}C) were purchased from common suppliers. TLC were performed on 60 F 254 silica-covered aluminum sheets. Eluted TLC were revealed using UV radiation (λ= 254 nm), vanillin, phosphomolybdic acid, or potassium permanganate solutions. Column chromatography were performed on silica gel 40-63 μm. Ozone gas for ozonolysis reactions was generated by an air cooled ozone generator. NMR spectra were recorded at 400 MHz for ^{1}H, 100 MHz for ^{13}C at 303 K, on samples dissolved in appropriate deuterated solvent. Used references were deuterated solvent signal for ^{1}H and ^{13}C. Chemical displacement values (δ) are expressed in parts per million (ppm), and coupling constants (*J*) in Hertz (Hz). Low-resolution mass spectra were performed on a UPLC/MS in positive electrospray ionization (ESI^+). Some mass spectra were performed on a quadripolar in electron impact (EI) (70 eV). High-resolution mass spectrometry (HRMS) analyses were performed at the « plateforme de spectrométrie de masse de l'IRS-UN à Nantes » on a LC-Q-Tof spectrometer in positive electrospray ionization (ESI^+) or at LABERCA (« laboratoire d'étude des résidus et contaminants dans les aliments ») on a LTQ-Orbitrap spectrometer in electrospray ionization. Melting points were measured on both a Kofler bench and a Tottoli system and are uncorrected. Optical rotations were determined with a polarimeter equipped with a Na lamp (589 nm) at a concentration c given in g/100mL in $CHCl_3$.

_S_-((1_S_,8_S_,9_S_,10_R_,13_S_,14_S_)-10,13-dimethyl-3,17-dioxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1_H_-cyclopenta[_a_]phenanthren-1-yl) ethanethioate (4a)[32]

To a solution of **ADD** (1.56 g, 5.5 mmol, 1.0 equiv) in 42 mL of a THF/MeOH/H_2O mixture (10:10:1), was added thioacetic acid (4.0 mL, 56.5 mmol, 10.3 equiv) and the reaction mixture was stirred under argon at room temperature. After all starting material was consumed (ca. 12 h, as indicated by TLC analysis), the reaction mixture was diluted with dichloromethane (100 mL) and washed three times with a saturated aqueous solution of $NaHCO_3$. Resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The resulting yellow solid was triturated with 5 mL of Et_2O for 3 h at room temperature and the solid was filtered and rinsed with a mixture of cyclohexane/Et_2O (3:1). The resulting white solid was dried under vacuum to furnish thioacetate **4a** (1.77 g, 89 %). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.73 (bs, 1H), 4.04 (t, 1H, *J* = 3.3 Hz), 2.95 (dd, 1H, *J* = 17.2, 3.7 Hz), 2.55 (ddd, 1H, *J* = 17.2, 2.0, 0.8 Hz), 2.44 (dd, 1H,

J = 19.1, 8.8 Hz), 2.41–2.36 (m, 2H), 2.29 (s, 3H,), 2.13–1.89 (m, 3H), 1.79 (dt, 1H, *J* = 12.9, 3.3 Hz), 1.69 (ddd, 1H, *J* = 22.0, 10.9, 3.3 Hz), 1.60–1.50 (m, 2H), 1.42-1.17 (m, 4H), 1.38 (s, 3H), 1.13–1.02 (m, 1H), 0.88 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 219.9 (C), 195.9 (C), 194.4 (C), 167.6 (C), 124.8 (CH), 50.9 (CH), 49.4 (CH), 48.8 (CH), 47.5 (C), 41.8 (CH_2), 41.6 (C), 35.8 (CH_2), 35.3 (CH), 32.4 (CH_2), 31.2 (CH_3), 31.1 (CH_2), 29.8 (CH_2), 21.8 (CH_2), 19.6 (CH_2), 19.5 (CH_3), 13.7 (CH_3). HRMS (ESI^+): calcd. for $C_{21}H_{28}O_3SNa$ [$(M+Na^+]$: 383.1657; Found: 383.1659. Mp 196-198 °C. $[\alpha]_D^{20}$ = 177.3 (c 0.23, $CHCl_3$).

(1*S*,8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-1-(phenylthio)-7,8,9,10,11,12,13,14,15,16-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,17(2*H*,6*H*)-dione (4b)[14]

To a solution of **ADD** (8.53 g, 30 mmol, 1.0 equiv) in anhydrous THF (150 mL) under argon was added thiophenol (30.6 mL, 300 mmol, 10.0 equiv) and sodium thiophenolate (19.81 g, 150 mmol, 5.0 equiv). The resulting mixture was stirred for 48 h at room temperature, diluted with 250 mL of dichloromethane and 100 mL of water were added. The organic layer was washed five times with 150 mL of a half-saturated aqueous solution of Na_2CO_3, and then washed with water and brine before dried over anhydrous Na_2SO_4. After filtration and evaporation, the pale yellow solid residue was triturated in a minimum of Et_2O for 5 h at room temperature. Filtration and high vacuum drying afforded the desired sulfide **4b** (9.80 g) in 83 % yield as a white crystalline solid. ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.41–7.38 (m, 2H), 7.33–7.25 (m, 3H), 5.82 (bd, 1H, *J* = 0.9 Hz), 3.54 (t, 1H, *J* = 3.25 Hz), 2.77 (dd, 1H, *J* = 16.9, 0.9 Hz), 2.59 (ddd, 1H, *J* = 16.9, 3.0, 0.9 Hz), 2.49 (ddd, 1H, *J* = 19.1, 9.1, 1.1 Hz), 2.46–2.42 (m, 2H), 2.12 (dt, 1H, *J* = 19.1 Hz, 9.1 Hz), 2.05–1.84 (m, 5H), 1.76 (ddd, 1H, *J* = 11.6, 11.6, 10.5 Hz), 1.64–1.33 (m, 4H), 1.38 (s, 3H), 1.29–1.13 (m, 1H), 0.93 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 220.2 (C), 196.1 (C), 165.7 (C), 134.0 (C), 133.7 (2CH), 129.4 (2CH), 128.1 (CH), 124.8 (CH), 54.5 (CH), 50.9 (CH), 48.0 (CH), 47.6 (C), 42.8 (C), 39.8 (CH_2), 35.9 (CH_2), 35.4 (CH), 32.5 (CH_2), 31.2 (CH_2), 29.8 (CH_2), 21.9 (CH_2), 20.0 (CH_3), 19.7 (CH_2), 13.9 (CH_3). HRMS (ESI^+): calcd. for $C_{25}H_{31}O_2S$ [$(M+H)^+$]: 395.2045; Found: 395.2029. Mp 188.1-189.3 °C. $[\alpha]_D^{20}$ = 97.7 (c 0.15, $CHCl_3$).

(1*S*,8*S*,9*S*,10*R*,13*S*,14*S*)-1-(ethylthio)-10,13-dimethyl-7,8,9,10,11,12,13,14,15,16-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,17(2*H*,6*H*)-dione (4c)

To a suspension of sodium hydride, 60 % dispersion in mineral oil, (200 mg, 5.0 mmol, 1.0 equiv) in anhydrous THF (10 mL) under argon was carefully added ethanethiol (0.6 mL, 20.0 mmol, 4.0 equiv) at 0 °C. A solution of **ADD** (1.42 g, 5.0 mmol, 1.0 equiv) in anhydrous THF (10 mL) was then added dropwise. The reaction mixture was stirred for 4 days at room temperature, and quenched with a saturated aqueous solution of NH_4Cl. The aqueous phase was extracted twice with ethyl acetate (50 mL) and the combined organic layers were washed sequentially with water and brine and then dried over anhydrous Na_2SO_4. After evaporation of volatiles under vacuum, NMR analysis of crude product showed the presence of a 1:1 mixture of starting material and expected product **4c**. The crude mixture was subjected to column chromatography on silica gel (cyclohexane:EtOAc, 7:3 to 6:4) to afford the sulfide compound **4c** as a white powder (745 mg, 43 %). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.76 (bd, 1H, J = 0.9 Hz), 3.17 (t, 3H, J = 3.3 Hz), 2.90 (dd, 1H, J = 16.8, 3.3 Hz), 2.71 (ddd, 1H, J = 16.8, 3.3, 0.9 Hz), 2.59–2.43 (m, 3H,), 2.41–2.37 (m, 2H), 2.10 (dt, 1H, J = 19.1, 9.0 Hz), 2.03–1.95 (m, 1H), 1.93-1.82 (m, 3H), 1.75–1.66 (m, 2H), 1.57 (tt, 1H, J = 12.4, 9.0 Hz), 1.48–1.31 (m, 3H), 1.36 (s, 3H), 1.22 (t, 3H, J = 7.4 Hz), 1.20–1.07 (m, 1H), 0.91 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 220.3 (C), 196.4 (C), 166.2 (C), 124.5 (CH), 50.9 (CH), 49.5 (CH), 47.8 (CH), 47.6 (C), 42.7 (C), 40.0 (CH_2), 35.9 (CH_2), 35.3 (CH), 32.6 (CH_2), 31.2 (CH_2), 29.7 (CH_2), 25.5 (CH_2), 21.9 (CH_2), 20.0 (CH_3), 19.6 (CH_2), 14.6 (CH_3), 13.8 (CH_3). HRMS (ESI^+): calcd. for $C_{21}H_{30}O_2SNa$ [$(M+Na)^+$]: 369.1864; Found: 369.1861. Mp 174.5-176.1 °C. $[\alpha]_D^{20}$ = 212.2 (c 0.115, $CHCl_3$).

(1*S*,8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-1-(phenylselanyl)-7,8,9,10,11,12,13,14,15,16-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,17(2*H*,6*H*)-dione (4d)

γ-Cyclodextrine (130 mg, 0.1 mmol, 10 mol%) was dissolved in water (15 mL) at room temperature. A solution of benzeneselenol (157 mg, 1.0 mmol, 1.0 equiv) in acetone (1 mL) and a solution of **ADD** (284 mg, 1.0 mmol, 1.0 equiv) in acetone (1 mL) were added dropwise. The reaction mixture was stirred for 6 h at room temperature. After this time, TLC analysis indicated the complete conversion of starting material. Dichloromethane (30 mL) was then added and the layers were partitioned into a separation funnel. Aqueous layer was re-extracted with 30 mL of dichloromethane and the combined organic layers were washed with brine. After drying over anhydrous Na_2SO_4 and evaporation under vacuum, the crude product was purified by column chromatography on silica gel (cyclohexane:EtOAc, 7:3) to yield the expected phenyl selenide

compound **4d** as a white powder (406 mg, 92%). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.55–7.52 (m, 2H), 7.33-7.25 (m, 3H), 5.82 (bd, 1H, J = 0.9 Hz), 3.61 (t, 1H, J = 3.3 Hz), 2.99 (dd, 1H, J = 17.2, 3.6 Hz), 2.67 (ddd, 1H, J = 17.2, 3.0, 0.9 Hz), 2.48 (dd, 1H, J = 19.2, 8.6 Hz), 2.44–2.40 (m, 2H), 2.12 (dt, 1H, J = 19.2, 8.6 Hz), 2.04-1.92 (m, 2H), 1.89–1.83 (m, 2H), 1.79–1.70 (m, 2H), 1.58 (tt, 1H, J = 12.2, 12.2 Hz), 1.54–1.31 (m, 3H), 1.36 (s, 3H), 1.25-1.08 (m, 1H), 0.92 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 220.1 (C), 196.3 (C), 166.2 (C), 136.0 (2CH), 129.4 (2CH), 128.8 (C), 128.4 (CH), 124.8 (CH), 51.6 (CH), 50.8 (CH), 50.3 (CH), 47.5 (C), 43.1 (C), 40.8 (CH_2), 35.9 (CH_2), 35.5 (CH), 32.5 (CH_2), 31.1 (CH_2), 29.8 (CH_2), 21.9 (CH_2), 19.8 (CH_2), 19.2 (CH_3), 13.9 (CH_3). HRMS (ESI^+): calcd for $C_{25}H_{31}O_2{}^{76}Se$ [$(M+H)^+$]: 439.1516; Found: 439.1507. Mp 192.1-193.8 °C. $[\alpha]_D^{20}$ = 128.4 (c 0.116, $CHCl_3$).

(*E*)-3-((3a*S*,5a*S*,6*R*,9a*S*,9b*S*)-3a,6-dimethyl-3,7-dioxododecahydro-1*H*-cyclopenta[*a*]naphthalen-6-yl)acrylic acid (5)

Phenyl sulfide **4b** (1.2 g, 3.04 mmol, 1.0 equiv) was dissolved in 100 mL of EtOAc under argon atmosphere. This solution was cooled to –78 °C and treated with an ozone bubbling for 1 h at –78 °C. The reaction mixture was then purged with an argon bubbling for 30 min and dimethyl sulfide (2.23 mL, 30.4 mmol, 10.0 equiv) was added and the mixture was allowed to reach room temperature. Stirring was maintained 14 h at room temperature and a 1 N solution of Na_2CO_3 (100 mL) was added. The biphasic solution was stirred vigorously for 15 minutes at room temperature and the two phases were separated. The aqueous layer was washed with ethyl acetate (100 mL) and then acidified to pH= 2 with a 37 % aqueous solution of hydrochloric acid. After extraction with dichloromethane (2 × 150 mL), the resulting organic phases were combined, washed with water (150 mL), brine (250 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and the crude expected acid **5** was obtained as white powder. The crude mixture was subjected to column chromatography on silica gel (AcOEt/MeOH, 10:0 to 8:2) to afford the acid **5** as a white powder (0.95g, 95%, E:Z = 92:8). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.05 (d, 1H, J = 16.1 Hz), 5.82 (d, 1H, J = 16.1 Hz), 2.66 (td, 1H, J = 14.4, 6.3 Hz), 2.50 (dd, 1H, J = 19.2, 8.6 Hz), 2.42 (ddd, 1H, J = 14.5, 4.4, 2.4 Hz), 2.19–1.86 (m, 4H), 1.83-1.79 (m, 1H), 1.68–1.23 (m, 7H), 1.35 (s, 3H), 0.93 (s, 3H), 0.90-0.77 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 219.7 (C), 211.8 (C), 170.6 (C), 154.1 (CH), 121.3 (CH), 54.7, 50.91, 50.83, 47.9, 37.7, 35.8, 34.4, 31.0, 30.4, 21.99, 21.93, 15.5 (CH_3), 13.9 (CH_3). HRMS

(ESI$^+$): calcd for $C_{18}H_{24}O_4Na$ [(M+Na)$^+$]: 327.1572; Found: 327.1570. Mp 201 °C. $[\alpha]_D^{20}$ = 130.3 (c 0.056, $CHCl_3$).

(3a*S*,5a*S*,6*S*,9a*S*,9b*S*)-3a,6-dimethyl-3,7-dioxododecahydro-1*H*-cyclopenta[*a*]naphthalene-6-carbaldehyde (1) [12a]

Procedure 1:

Phenyl sulfide **4b** (1.2 g, 3.04 mmol, 1.0 equiv) was dissolved in 100 mL of EtOAc under argon atmosphere. This solution was cooled to –78 °C and treated with an ozone bubbling for 1 h at –78 °C. The reaction mixture was then purged with an argon bubbling for 30 min and allowed to reach room temperature. The resulting yellow solution was stirred for 1 h at 25 °C. The mixture was then cooled to –78 °C and treated with ozone bubbling for 1 h. After a purge by argon bubbling, dimethyl sulfide (2.23 mL, 30.4 mmol, 10.0 equiv) was added and the mixture was allowed to reach room temperature. Stirring was maintained 14 h at room temperature and water (100 mL) was added. The organic phase was washed with brine and dried over anhydrous sodium sulfate and concentrated under vacuum. The yellow oily residue was subjected to column chromatography on silica gel (cyclohexane:EtOAc, gradient 7:3 to 6:4) to afford the expected diketo-aldehyde **1** as a white crystalline solid in 43 % yield.

Procedure 2:

Preparation of silica gel supported sodium periodate: Sodium periodate (25.7 g, 120 mmol) was dissolved in 50 mL of hot water (80 °C). To this hot solution was added silica gel (230-400 mesh, 100 g) with vigorous stirring and shaking. The resultant powder and free-flowing silica gel coated with $NaIO_4$ was placed in a mortar and was finely grounded with a pestle. The resulting fine powdered solid can be stored in a closed bottle for months. *Oxidative cleavage:* Compound **10** (5.53 g, 18.78 mmol) was dissolved in dichloromethane (240 mL) and silica gel-supported sodium periodate (50 g) was added. The reaction mixture was stirred vigorously at room temperature for 4 h. At this point, TLC analysis showed complete conversion of starting material. Dichloromethane was removed under vacuum and the resulting crude product adsorbed on silica gel was subjected to a short column chromatography on silica gel (cyclohexane:EtOAc, 65:35) to afford diketo-aldehyde **1** as a white crystalline solid (4.53 g, 92 %). ^{1}H NMR

(400 MHz; $CDCl_3$) δ_H 9.53 (s, 1H), 2.58 (dt, 1H, *J* = 14.4, 6.5 Hz), 2.51 (dd, 1H, *J* = 19.0, 8.8 Hz), 2.42 (ddd, 1H, *J* = 14.4, 4.5, 2.4 Hz), 2.21–2.00 (m, 3H), 1.96–1.80 (m, 3H), 1.64 (tt, 1H, *J* = 12.3, 8.9 Hz), 1.55–1.38 (m, 3H), 1.35–1.25 (m, 1H), 1.30 (s, 3H), 1.24–1.16 (m, 1H), 0.93 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 219.3 (C), 212.2 (C), 201.1 (C), 62.5 (C), 50.7 (CH), 47.9 (C), 46.1 (CH), 38.1 (CH_2), 35.6 (CH_2), 34.0 (CH), 30.9 (CH_2), 30.0 (CH_2), 21.9 (CH_2), 21.8 (CH_2), 13.9 (CH_3), 12.6 (CH_3). HRMS (ESI^+): calcd for $C_{16}H_{22}O_3Na$ [$(M+Na)^+$]: 285.1467; Found: 285.1465. Mp 199.5-201.0 °C. $[\alpha]_D^{20}$ = 75.6 (c 0.348, $CHCl_3$).

3-((3a*S*,5a*S*,6*R*,9a*R*,9b*S*)-3a,6-dimethyl-3,7-dioxododecahydro-1*H*-cyclopenta[*a*]naphthalen-6-yl)propanoic acid (6)[33]

In a 2L round-bottom flask was placed a solution of **AD** (11.45 g, 40.0 mmol, 1.0 equiv) in *i*-PrOH (460 mL). A solution of sodium carbonate (5.09 g, 48.0 mmol, 1.2 equiv) in water (40 mL) was added and the mixture was heated at 70 °C. In a separate flask, potassium permanganate (379 mg, 2.4 mmol, 6 mol%) and sodium periodate (48.77 g, 228.0 mmol, 5.7 eq.) were dissolved in hot water (360 mL, 70 °C) and this purple solution was carefully and slowly added to the reaction flask. The resulting mixture was vigorously stirred at 70 °C until TLC analysis revealed complete consumption of starting material (ca. 4 h). The reaction mixture was cooled to room temperature and filtered through a pad of Celite®. The pad was rinsed with DCM (100 mL) and then with a 0.5 N aqueous solution of NaOH (200mL). Dichloromethane and *i*-PrOH were removed under reduced pressure and the resulting basic aqueous solution was washed with dichloromethane (2 × 200 mL) and then acidified to pH= 2 with a 37 % aqueous solution of hydrochloric acid. After extraction with dichloromethane (2 × 250 mL), the resulting organic phases were combined, washed with water (150 mL), brine (250 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and the expected acid **6** (12.24 g, white foam) was used in the next step without further purification. An analytical sample was purified by column chromatography on silica gel (cyclohexane:EtOAc, 3:7 to 1:9). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 10.90 (bs, 1H), 2.53 (td, 1H, *J* = 14.4 Hz, 6.3 Hz), 2.42 (dd, 1H, *J* = 19.2, 8.7 Hz), 2.32–2.24 (m, 2H), 2.21-2.13 (m, 1H), 2.10–2.00 (m, 3H), 1.97-1.77 (m, 3H), 1.60–1.44 (m, 4H), 1.31–1.18 (m, 4H), 1.08 (s, 3H, H19), 0.87 (s, 3H, H18). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 220.2 (C), 214.0 (C), 179.3 (C), 50.7 (CH), 50.4 (C), 48.0 (CH), 47.6 (C), 37.7 (CH_2), 35.7

(CH_2), 34.4 (CH), 30.9 (CH_2), 29.9 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 21.8 (CH_2), 20.7 (CH_2), 20.3 (CH_3), 13.7 (CH_3). HRMS (ESI^+): calcd for $C_{18}H_{26}O_4Na$ [$(M+Na)^+$]: 329.1729; Found: 329.1741. Mp 107.8-109.7 °C. $[\alpha]_D^{20}$ = 105.1 (c 0.216, $CHCl_3$).

3-((3a'S,5a'S,6'R,9a'R,9b'S)-3a',6'-dimethyldecahydro-5'H-dispiro[[1,3]dioxolane-2,3'-cyclopenta[a]naphthalene-7',2''-[1,3]dioxolan]-6'-yl)propanoic acid (7)

The crude diketo-acid **6** (12.24 g, 39.95 mmol, 1.0 equiv) was dissolved in dichloromethane (100 mL) under argon. Ethylene glycol (6.7 mL, 120.0 mmol, 3.0 equiv), triethyl orthoformate (20.0 mL, 120.0 mmol, 3.0 equiv) and *p*-toluenesulfonic acid monohydrate (761 mg, 4.0 mmol, 0.1 equiv) were added and the reaction mixture was heated at reflux for 16 h and then cooled to room temperature. Water (100 mL) was added and the organic layer was separated. Aqueous layer was extracted twice with DCM (2 × 100 mL) and the combined organic layers were washed with brine (100 mL) and dried over Na_2SO_4. After filtration and solvent evaporation under vacuum, the residue was dissolved in methanol (100 mL). A 2 N aqueous solution of NaOH (50 mL) was added and the mixture was heated at reflux with vigorous stirring for 1.5 h and then cooled room temperature. Water (250 mL) was added and methanol was evaporated under vacuum. The aqueous layer was washed twice with DCM (2 × 200 mL) and acidified to pH= 2–3 with a 37 % aqueous solution of hydrochloric acid. After two extractions with DCM (2 × 200 mL) the obtained organic phases were combined, washed with water (200 mL), brine (250 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum, and the expected product **7** (13.7 g, 87 %, white foam) was used in the next step without further purification. An analytical sample was purified by column chromatography on silica gel (cyclohexane:EtOAc, 6:4). ^1H NMR (400 MHz; C_6D_6) δ_H 3.65–3.30 (m, 8H), 2.9–2.7 (m, 1H), 2.6–2.4 (m, 1H), 2.3–2.0 (m, 2H), 1.95–1.05 (m, 15H), 0.93 (s, 3H), 0.72 (s, 3H). ^{13}C NMR (100 MHz; C_6D_6) δ_C 181.8 (C3), 119.5 (C), 113.9 (C), 65.3 (CH_2), 64.7 (CH_2), 64.2 (CH_2), 64.0 (CH_2), 50.2 (CH), 46.4 (C), 46.1 (CH), 43.2 (C), 35.4 (CH), 34.7 (CH_2), 31.1 (CH_2), 31.0 (CH_2), 30.4 (CH_2), 30.0 (CH_2), 27.7 (CH_2), 23.1 (CH_2), 21.6 (CH_2), 18.5 (CH_3), 14.8 (CH_3). HRMS (ESI^+): calcd for $C_{22}H_{34}O_6Na$ [$(M+Na)^+$]: 417.2253; Found: 417.2246. Mp 92.5 °C. $[\alpha]_D^{20}$ = 12.9 (c 0.14, $CHCl_3$).

(3a'*S*,5a'*S*,6'*R*,9a'*S*,9b'*S*)-3a',6'-dimethyl-6'-vinyldecahydro-5'*H*-dispiro[[1,3]dioxolane-2,3'-cyclopenta[*a*]naphthalene-7',2''-[1,3]dioxolane] (8)

Diketal **7** (13.7 g, 34.73 mmol, 1.0 equiv) was dissolved in DMPU (70 mL) under argon and acetic anhydride (3.61 mL, 38.20 mmol, 1.1 equiv) was added. The resulting solution was stirred at room temperature for 10 min and triphenylphosphine (6.83 g, 26.04 mmol, 0.75 equiv) was added followed by palladium dichloride (185 mg, 1.04 mmol, 3 mol%). The reaction mixture was degassed (vacuum/argon, three times) and heated to 250 °C. The rate of decarbonylation is followed by liberation of CO (reaction begins about 245 °C with apparition of an intense red coloration). Heating was maintained 75 min at 250 °C after beginning of bubbling and cooled to room temperature. The resulting dark mixture was filtered through a pad of Celite® and the pad was rinsed with Et_2O (300 mL). Water (250 mL) was added to the filtrate and layers were separated. Aqueous layer was re-extracted by Et_2O (300 mL) and combined organic layers were washed with a 2 N aqueous solution of NaOH (250 mL), water (200 mL), brine (200 mL) and dried over Na_2SO_4. Solvent was evaporated to afford 15.7 g of crude product **8**, which was used in the next step without further purification. An analytical sample was purified by column chromatography on silica gel (cyclohexane:EtOAc, 9:1). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.83 (dd, J = 17.7, 11.0 Hz, 1H), 5.09 (dd, J = 11.0, 1.7 Hz, 1H), 5.03 (dd, J = 17.7, 1.7 Hz, 1H), 3.95–3.79 (m, 8H), 1.98 (ddd, J = 14.2, 11.4, 2.8 Hz, 1H), 1.83–1.17 (m, 14H), 1.10 (s, 3H), 0.83 (d, J = 0.5 Hz, 3H). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 144.0 (CH), 119.4 (C), 114.6 (CH_2), 113.0 (C), 65.4 (CH_2), 65.31 (CH_2), 65.27 (CH_2), 64.7 (CH_2), 50.0 (CH), 48.0 (C), 47.7 (CH), 46.4 (C), 34.8 (CH), 34.4 (CH_2), 31.4 (CH_2), 30.7 (CH_2), 27.7 (CH_2), 22.7 (CH_2), 22.0 (CH_2), 14.6 (CH_3), 13.5 (CH_3). HRMS (ESI$^+$): calcd for $C_{21}H_{32}O_4Na$ [$(M+Na)^+$]: 371.2198; Found: 371.2186. Mp 153.3-156.7 °C. $[\alpha]_D^{20}$ = 25.4 (c 0.063, $CHCl_3$).

(3a*S*,5a*S*,6*R*,9a*S*,9b*S*)-3a,6-dimethyl-6-vinyloctahydro-1*H*-cyclopenta[*a*]naphthalene-3,7(2*H*,3a*H*)-dione (9)

Crude vinylic compound **8** (15.7 g) obtained in the previous step was placed in an AcOH:H_2O mixture (1:1, 350 mL) under argon atmosphere. The reaction mixture was heated at reflux temperature for 1 h and then cooled to room temperature. At this point TLC showed complete conversion of starting material. The half

initial volume of solvent was removed under vacuum and a saturated aqueous solution of sodium carbonate (800 mL) was slowly added via a dropping funnel at 0 °C. The resulting aqueous solution was extracted three times with Et_2O (3 × 500 mL) and the combined organic layers were washed with water (300 mL) and brine (300 mL) before drying over Na_2SO_4. Solvent was removed under vacuum and the yellow solid residue was subjected to a short column chromatography on silica gel (cyclohexane:EtOAc, 75:25) to afford expected diketo-olefin **9** as a white crystalline solid (6.11 g). (Overall yield from AD: 59 %). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 5.78 (dd, 1H, *J* = 17.6, 10.9 Hz), 5.25 (dd, 1H, *J* = 10.9, 1.0 Hz), 5.03 (dd, 1H, *J* = 17.6, 1.0 Hz), 2.62 (td, 1H, *J* = 14.3, 6.3 Hz), 2.48 (ddd, 1H, *J* = 19.1, 9.0, 0.8 Hz), 2.38 (ddd, 1H, *J* = 14.3, 4.5, 2.5 Hz), 2.15–2.06 (m, 2H), 2.02–1.95 (m, 1H), 1.91 (bdq, 1H, *J* = 10.7, 3.6 Hz), 1.79 (ddd, 1H, *J* = 13.0, 3.8, 2.8 Hz), 1.61 (tt, 1H, *J* = 12.2, 9.0 Hz), 1.55–1.20 (m, 5H), 1.26 (s, 3H), 0.92 (d, 3H, *J* = 0.6Hz). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 219.9 (C), 213.6 (C), 141.4 (CH), 114.9 (CH_2), 54.6 (C), 51.1 (CH), 51.0 (CH), 48.0 (C), 37.8 (CH_2), 35.8 (CH_2), 34.5 (CH), 31.1 (CH_2), 30.4 (CH_2), 21.9 (CH_2), 21.7 (CH_2), 15.2 (CH_3), 13.9 (CH_3). HRMS (ESI$^+$): calcd. for $C_{17}H_{24}O_2Na$ [$(M+Na)^+$]: 283.1674; Found: 283.1665. Mp 142.3-143.9 °C. $[\alpha]_D^{20}$ = 88.2 (c 0.11, $CHCl_3$).

(3a*S*,3b*S*,5a*S*,8a*S*,8b*S*)-3,10a-dihydroxy-3a,5a-dimethyldodecahydro-2*H*-cyclopenta[5,6]naphtho[2,1-*b*]furan-6(7*H*)-one (10)

In a 250 mL round-bottom flask, compound **9** (6.37 g, 24.46 mmol, 1.0 equiv) was dissolved in acetone (100 mL) and water (50 mL) was added followed by 2,6-lutidine (5.7 mL, 48.93 mmol, 2.0 equiv), *N*-methylmorpholine-*N*-oxide (11.46 g, 97.86 mmol, 4.0 equiv) and a 4% aqueous solution of osmium tetroxide (7.8 mL, 1.22 mmol, 5 mol%). The flask was hermetically closed under an argon atmosphere and the solution was stirred for 60 h at room temperature. At this point, TLC analysis showed complete conversion of starting material. The reaction mixture was poured into a saturated aqueous solution of sodium thiosulfate (100 mL) and the resulting mixture was stirred vigorously for 30 min. Acetone was removed under vacuum and the resulting aqueous phase was extracted four times with dichloromethane (4 × 200 mL). The combined organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure and the residue was subjected to column chromatography on silica gel (cyclohexane:EtOAc, 2:8) to afford 5.54 g (76 % yield) of a mixture of 2 diastereoisomers of hemiketal **10** and 2 diastereoisomers of open form of the

expected dihydroxylated compound as viscous oil. The following spectral data refers to the major isomer hemiketal form (ca. 80 % in $CDCl_3$). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.24 (dd, 1H, J = 10.4 5.4 Hz), 4.03 (bdd, 1H, J = 9.0, J = 5.4 Hz), 3.92 (dd, 1H, J = 10.4, 1.0 Hz), 3.19 (s, 1H), 3.06 (d, 1H, J = 9.0 Hz), 2.45 (bdd, 1H, J = 19.6, 8.6 Hz), 2.07 (dt, 1H, J = 19.6, 9.0 Hz), 1.98–1.91 (m, 2H), 1.83 (bdt, 1H, J = 13.0, 3.4 Hz), 1.69–1.41 (m, 6H), 1.27–1.18 (m, 2H), 1.18–1.12 (m, 1H), 1.12 (s, 3H), 0.89 (s, 3H), 0.75 (bdt, 1H, J = 11.1, 4.6 Hz). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 220.6 (C), 107.2 (C), 77.3 (CH), 74.3 (CH_2), 51.1 (CH), 50.9 (C), 47.9 (C), 46.0 (CH), 35.9 (CH_2), 34.7 (CH), 31.69 (CH_2), 31.66 (CH_2), 26.6 (CH_2), 22.3 (CH_2), 21.9 (CH_2), 13.9 (CH_3), 10.1 (CH_3). HRMS (ESI^+): calcd. for $C_{17}H_{26}O_4Na$ [$(M+Na)^+$]: 317.1729; Found: 317.1718.

[^{13}C2]-acetylimidazole (12)[34]

To a solution of 1H-imidazole (2.79 g, 40.99 mmol, 2.0 equiv) in a THF:Et_2O mixture (25 mL, 1:1) at 0 °C, was slowly added [^{13}C2]-acetyl chloride (1.65 g, 20.49 mmol, 1.0 equiv) and the resulting suspension was vigorously stirred for 2 h. The salts were removed by filtration of the mixture on Büchner, washed twice with 30 mL of diethyl ether and the solution was evaporated under vacuum. The white solid was dried over P_2O_5 under vacuum to yield 91 % (2.09 g) of the expected labeled compound **12**, which was used in the next step without further purification. ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.13 (bs, 1H), 7.46 (bs, 1H), 7.10 (bs, 1H), 2.60 (dd, 3H, $J_{C\text{-}H}$ = 130.1 Hz, $J_{C\text{-}H}$ = 6.7 Hz). ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 166.5 (d, $J_{C\text{-}C}$ = 53.3 Hz, ^{13}C), 136.5 (CH), 131.3 (CH), 116.3 (CH), 23.0 (d, $J_{C\text{-}C}$ = 53.3 Hz, $^{13}CH_3$).

[^{13}C]-methyltriphenylphosphonium iodide (13)[35]

Triphenylphosphine (13,9 g, 52.8 mmol, 1.5 equiv) was solubilized in anhydrous toluene (50 mL) under argon and the solution was cooled to 0 °C. ^{13}C-Iodomethane (5.0 g, 35.0 mmol, 1.0 equiv) was quickly added and the flask was hermetically closed. The solution was stirred for 12 h at room temperature and the resulting white suspension was filtered on Büchner. The white solid was rinsed three times with 50 mL of anhydrous toluene and then dried under vacuum over P_2O_5 to afford ^{13}C-labeled phosphonium iodide **13** in quantitative yield (14.1 g). ^{1}H NMR (400 MHz; DMSO d_6) δ_H 7.93-7.86 (m, 3H), 7.80-7.74 (m, 12H), 3.17 (dd, 3H, $J_{C\text{-}H}$ = 134.9 Hz, $J_{P\text{-}H}$ = 14.6 Hz). ^{31}P NMR (162 MHz, DMSO d_6): δ_P 22.65 (d, $J_{C\text{-}P}$ = 55.5 Hz). ^{13}C

NMR (100 MHz; DMSO d_6) δ_C 134.8 (d, $J_{C\text{-}P}$ = 2.9 Hz, 3CH), 133.2 (d, $J_{C\text{-}P}$ = 10.8 Hz, 3CH), 130.1 (d, $J_{C\text{-}P}$ = 12.7 Hz, 3CH), 119.9 (d, $J_{C\text{-}P}$ = 88.1 Hz, 3C), 7.31 (d, $J_{C\text{-}P}$ = 55.5 Hz, $^{13}CH_3$).

[1,2,3-^{13}C3]-1-(triphenylphosphoranylidene)-2-propanone (2)[36,12a]

To a suspension of [^{13}C]-methyltriphenylphosphonium iodide **13** (2.03 g, 5.0 mmol, 1.0 equiv) in anhydrous THF (50 mL) under argon, was added dropwise a 1 N solution of LiHMDS in THF (5.0 mL, 5.0 mmol, 1.0 equiv) at room temperature. The resulting yellow solution was stirred for 30 min at room temperature and then cooled to -78 °C. 1 N Solution of LiHMDS in THF (5.0 mL, 5.0 mmol, 1.0 equiv) was quickly added followed by a slow addition of a solution of [^{13}C2]-acetylimidazole **12** (561 mg, 5.0 mmol, 1.0 equiv) in anhydrous THF (10 mL). The reaction mixture was stirred for 4 h at 0 °C and then poured to a 2 N aqueous solution of hydrochloric acid. The aqueous layer was washed with diethyl ether (2 × 100 mL) and then poured to a 10 N aqueous solution of NaOH (200 mL). The aqueous layer was extracted twice with 200 mL of a DCM:Et_2O mixture (1:2) and the combined organic layers were dried over anhydrous Na_2SO_4. Solvents were removed under reduced pressure to afford 1.4 g (90 %, purity = 93 % (UPLC-MS data)) of the expected stabilized ylide **2** as pale yellow solid. This compound was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO d_6): δ_H 7.68-7.62 (m, 5H), 7.57-7.52 (m, 3H), 7.47-7.43 (m, 5H), 3.70 (bd, 1H, $J_{C\text{-}H}$ = 165.7 Hz), 2.10 (ddt, 3H, $J_{C\text{-}H}$ = 125.8 Hz, J = 5.1, 2.1 Hz). ^{31}P NMR (162 MHz, DMSO d_6): δ_P (ppm) 14.3 (d, $J_{C\text{-}P}$ = 107.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 191.0 (ddd, $J_{C\text{-}C}$ = 64.5 Hz, $J_{C\text{-}C}$ = 41.5 Hz, $J_{C\text{-}P}$ = 2.1 Hz, ^{13}C), 133.2 (d, $J_{C\text{-}P}$ = 10.1 Hz, 3CH), 132.1 (d, $J_{C\text{-}P}$ = 2.9 Hz, 3CH), 128.9 (d, $J_{C\text{-}P}$ = 12.2 Hz, 3CH), 127.2 (d, $J_{C\text{-}P}$ = 90.6 Hz, 3C), 52.0 (ddd, $J_{C\text{-}P}$ = 107.6 Hz, $J_{C\text{-}C}$ = 64.4 Hz, $J_{C\text{-}C}$ = 19.2 Hz, ^{13}CH), 28.6 (ddd, $J_{C\text{-}C}$ = 41.5 Hz, $J_{C\text{-}C}$ = 19.3 Hz, $J_{C\text{-}P}$ = 15.8 Hz, $^{13}CH_3$).

[2,3,4-^{13}C3]-(3a*S*,5a*S*,6*R*,9a*S*,9b*S*)-3a,6-dimethyl-6-((*E*)-3-oxobut-1-en-1-yl)octahydro-1*H*-cyclopenta[*a*]naphthalene-3,7(2*H*,3a*H*)-dione (11)[12a]

To a solution of diketo-aldehyde **1 (**1.22 g, 4.67 mmol, 1.0 equiv) in degassed *p*-xylene (70 mL) was added [1,2,3-^{13}C3]-1-(triphenylphosphoranylidene)-2-propanone **2** (1.50 g, 4.67 mmol, 1.0 equiv). The resulting suspension was heated to reflux for 7 days. After this time, the mixture was allowed to reach room temperature and 50 mL of Et_2O were added. The solution was filtered through a pad of Celite® to remove

precipitated solids and then concentrated under vacuum. The dark-brown residue was subjected to column chromatography on silica gel (cyclohexane:EtOAc, gradient 7:3 to 6:4) to afford the corresponding (*E*)-enone **11** as a pale yellow oil (1.06 g, 75 %). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 6.75 (ddd, 1H, J = 16.5 Hz, J_{C-H} = 6.7 Hz, J_{C-H} = 1.4 Hz), 5.94 (ddt, 1H, J = 16.5 Hz, J_{C-H} = 157,6 Hz, J_{C-H} = 2.0 Hz), 2.62 (dt, 1H, J = 14.4, 6.3 Hz), 2.43 (dd, 1H, J = 19.0, 9.0 Hz), 2.34 (bd, 1H, J = 14.4 Hz), 2.20 (ddd, 3H, J_{C-H} = 127.4 Hz, J_{C-H} = 5.9 Hz, J_{C-H} = 1.1 Hz), 2.13-1.84 (m, 4H), 1.73 (bd, 1H, J = 13.2 Hz), 1.57 (ddd, 1H, J = 21.6 Hz, J = 11.9 Hz, J = 9.3 Hz), 1.48–1.11 (m, 6H), 1.27 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 219.4 (C), 211.9 (d, J_{C-C} = 1.6 Hz, ^{13}C), 198.0 (dd, J_{C-C} = 52.8 Hz, J_{C-C} = 42.4 Hz, ^{13}C), 150.3 (dd, J_{C-C} = 70.1 Hz, J_{C-C} = 1.3 Hz, ^{13}CH), 131.0 (dd, J_{C-C} = 52.8 Hz, J_{C-C} = 14.8 Hz, ^{13}CH), 54.4 (d, J_{C-C} = 5.9 Hz, C), 50.8 (d, J_{C-C} = 1.4 Hz, CH), 50.7 (CH), 47.7 (C), 37.5 (CH_2), 35.6 (CH_2), 34.2 (CH), 30.8 (CH), 30.2 (CH_2), 27.1 (dd, J_{C-C} = 42.4 Hz, J_{C-C} = 14.8 Hz, $^{13}CH_3$), 21.8 (CH_2) 21.7 (CH_2), 15.4 (d, J_{C-C} = 3.4 Hz, CH_3), 13.8 (CH_3). HRMS (ESI$^+$): calcd. for $C_{16}{}^{13}C_3H_{26}O_3Na$ [$(M+Na)^+$]: 328.1880; Found: 328.1884. $[\alpha]_D{}^{20}$ = 171 (c 1.1, $CHCl_3$).

[2,3,4-^{13}C3]-(8*R*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-7,8,9,10,11,12,13,14,15,16-decahydro-3*H*-cyclopenta[*a*]phenanthrene-3,17(6*H*)-dione (^{13}C3-ADD)[37]

To a solution of enone **11** (60 mg, 0.20 mmol, 1.0 equiv) in anhydrous toluene (2 mL) was added thioacetic acid (17 µL, 0.24 mmol, 1.2 equiv) and PTSA.H_2O (11 mg, 0.06 mmol, 0.3 equiv). The reaction mixture was stirred at 75 °C for 18 h. The solution was cooled to room temperature and DBU (60 µL, 0.39 mmol, 2.0 equiv) was added. The resulting solution was stirred for 30 min at room temperature and a saturated aqueous solution of $NaHCO_3$ (10 mL) was added. The aqueous layer was extracted with dichloromethane (2 × 10 mL) and the combined organic layers were washed with a saturated aqueous solution of NH_4Cl (15 mL), water (10 mL), brine (10 mL) and then dried over anhydrous Na_2SO_4. Solvents were removed under reduced pressure and the residue was subjected to column chromatography on silica gel (cyclohexane:EtOAc, gradient 7:3 to 6:4) to afford **^{13}C3-ADD** as a white crystalline solid (42 mg, 75 %). ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.02 (t, 1H, J = 10.1 Hz), 6.20 (bdd, 1H, J = 9.6 Hz, J_{C-H} = 164.8 Hz), 6.05 (bd, 1H, J_{C-H} = 161.5 Hz), 2.55–2.38 (m, 3H), 2.14–2.04 (m, 2H), 1.96 (dddd, 1H, J = 12.4, 8.8, 5.8, 0.7 Hz), 1.87–1.53 (m, 6H), 1.32–1.19 (m, 2H), 1.25 (s, 3H), 1.19–1.07 (m, 2H), 0.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 219.8 (C),

186.3 (dd, J = 54.0, 52.3 Hz, ^{13}C), 168.2 (C), 155.2 (CH), 127.8 (m, ^{13}CH), 124.2 (m, ^{13}CH), 52.4 (CH), 50.5 (CH), 47.7 (C), 43.5 (C), 35.7 (CH_2), 35.2 (CH), 32.6 (CH_2), 32.4 (CH_2), 31.3 (CH_2), 22.2 (CH_2), 22.0 (CH_2), 18.8 (CH_3), 13.9 (CH_3). HRMS of non-labeled **ADD** (ESI^+): calcd for $C_{19}H_{24}O_2Na$ [$(M+Na)^+$]: 307.1674, Found: 307.1665. HRMS of **^{13}C3-ADD** (ESI^+): calcd for $C_{16}{}^{13}C_3H_{24}O_2Na$ [$(M+Na)^+$]: 310.1775, Found: 310.1764. Mp 144.5-145.0 °C. $[\alpha]_D^{20}$ = 113.3 (c 1.02, $CHCl_3$).

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Supporting Information

Synthesis and experimental part of compounds **12**, **13** and **2**, ^{1}H, ^{13}C and ^{31}P NMR spectra of **4a-d**, **5**, **1**, **6-11**, **^{13}C3-11**, 1,4-androstadien-3,17-dione and 2,3,4-^{13}C3-1,4-androstadien-3,17-dione, **12**, **13** and **2**, as well as a proposed reaction pathway for the formation of keto-aldehyde **1** from **4b** (Scheme 1) and an ORTEP drawing of thiophenol adduct **4b** (Figure 1.) are presented in supporting information. This material is available free of charge via the Internet at http://pubs.acs.org.

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References and notes

[1] For recent works in this field, see: (a) Piotrowski, D. W. *J. Med. Chem*. **2012**, *55*, 7957−7966. (b) Martinez Botella, G.; Salituro, F. G.; Harrison, B. L.; Beresis, R. T.; Bai, Z.; Chen, K.; Belfort, G. M.; Loya, C. M.; Ackley, M. A.; Grossman, S. J. ; Hoffmann, E.; Jiag, S.; Wang, J.; Doherty, J. J.; Robichaud, A. J. *J. Med. Chem*. **2015**, *58*, 3500−3511. (c) Martinez Botella, G.; Salituro, F. G.; Harrison, B. L.; Beresis, R. T.; Bai, Z.; Blanco, M.-J.; Belfort, G. M.; Dai, J.; Loya, C. M.; Ackley, M. A.; Althaus, A. L.; Grossman, S. J.; Hoffmann, E.; Doherty, J. J.; Robichaud, A. J. *J. Med. Chem*. **2017**, *60*, 7810−7819, and cited literature.

[2] Incerti, M.; Russo, S.; Callegari, D.; Pala, D.; Carmine Giorgio, Zanotti, I.; Barocelli, E.; Vicini, P.; Vacondio, F.; Rivara, S.; Castelli, R.; Tognolini, M.; Lodola, A. *J. Med. Chem*. **2017**, *60*, 787−796.

[3] (a) Aqai, P.; Cevik, E.; Gerssen, A.; Haasnoot, W.; Nielen, M. W. F. *Anal. Chem*. **2013**, *85*, 3255–3262. (b) Thevis, M.; Kuuranne, T.; Geyera, H.; Schänzer, W. *Drug Test. Analysis* **2014**, *6*, 164–184. (c) Onakomaiya, M. M.; Henderson, L. P. *Psychopharmacology* **2016**, *233*, 549-569. (d) Dumont, Q.; Bárcenas, M.; Dossmann, H.; Bailloux, I.; Buisson, C.; Mechin, N.; Molina, A.; Lasne, F.; Rannulu, N. S.; Cole, R. B. *Anal. Chem*. **2016**, *88*, 3585–3591 and cited literature.

[4] Yinga, G.-G. ; Kookanaa, R. S.; Ru, Y.-J. *Environ. Int.* **2002**, *28*, 545–551.

[5] For a recent example of quantification using stable isotope dilution, see: Nakanishi, A.; Fukushima, Y.; Miyazawa, N.; Yoshikawa, K.; Maeda, T.; Kurobayashi, Y. *J. Agric. Food Chem*. **2017**, *65*, 5026–5033 and cited literature.

[6] Esaki, H.; Ito, N.; Sakai, S.; Maegawa, T.; Monguchi, Y.; Sajiki, H. *Tetrahedron* **2006**, *62*, 10954–10961 and cited literature.

[7] For excellent reviews in this field, see: (a) Atzrodt, J.; Derdau, V.; Fey, T.; Zimmermann, J. *Angew. Chem. Int. Ed.* **2007**, *46*, 7744–7765. (b) Ito, N.; Watahiki, T.; Maesawa, T.; Maegawa, T.; Sajiki, H. *Synthesis* **2008**, 1467–1478. (c) Lockley, W. J. S.; Heys, J. R. *J. Label Compd. Radiopharm*. **2010**, *53*, 635–644. (d) Sawama, Y.; Monguchi, Y.; Sajiki, H. *Synlett* **2012**, 959–972.

[8] Derdau, V.; Atzrodt, J. *Synlett* **2006**, 1918–1922.

[9] For reviews on this topic, see: (a) Chapelon, A.-S.; Moraléda, D.; Rodriguez, R.; Ollivier, C.; Santelli, M. *Tetrahedron* **2007**, *63*, 11511–11616. (b) Hanson, J. R. *Nat. Prod. Rep*. **2010**, *27*, 887–899.

[10] For representative examples, see: (a) Leontjev, A. E.; Vasiljeva, L. L.; Pivnitsky, K. K. *Russ. Chem. Bull.* **2004**, *53*, 703–708. (b) Furuta, T.; Suzuki, A.; Matsuzawa, M.; Shibasaki, H.; Kasuya, Y. *Steroids* **2003**, *68*, 693–703. (c) Vasiljeva, L. L.; Detain, P. M.; Kochev, D. M.; Lapitskaya, M. A.; Pivnitsky, K. K. *Russ. Chem. Bull.,* **1999,** *48*, 593–595. (d) Ramachary, D. B.; Sakthidevi, R.; Reddy, P. S. *RSC Adv.* **2013***, 3,* 13497–13506. (e) Yasui, N.; Mayne, C. G.; Katzenellenbogen, J. A. *Org. Lett.* **2015**, *17*, 5540–5543. (f) Kawahara, R.; Fujita, K.-I.; Yamaguchi, R. *Angew. Chem. Int. Ed.* **2012**, *51*, 12790–12794. (g) Zheng, D.-Q.; Jing, Y.; Zheng, B.-Y.; Ye, Y.-F.; Xu, S.; Tian, W.-S.; Ma, H.-Y.; Ding, K. *Tetrahedron*, **2016**, *72*, 2164–2169. (h) Rao, P. N.; Cessac, J. W.; Tinley, T. L.; Mooberry, S. L. *Steroids*, **2002**, *67*, 1079–1089.

[11] (a) Tochtrop, G. P.; DeKoster, G. T.; Cistola, D. P.; Covey, D. F. *J. Org. Chem.* **2002**, *67*, 6764–6771. (b) Fontana, E. ; Pignatti, A. ; Giribone, D.; Di Salle, E. *Steroids* **2008**, *73*, 760–764.

[12] (a) Kockert, K.; Vierhapper, F. W. *Tetrahedron* **2000**, *56*, 9967–9974. For preliminary studies, see: (b) de Avellar, I. G. J.; Vierhapper, F. W. *Tetrahedron* **2000**, *56*, 9957–9965.

[13] (a) Gentles, M. J.; Moss, J. B.; Herzog, H. L.; Hershberg, E. B. *J. Am. Chem. Soc*. **1958**, *80*, 3702–3705. (b) Hanson, J. R.; Reese, P. B.; Sadler, I. H. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2937–2939.

[14] Brueggemeier, R. W.; Floyd, E. E.; Counsell, R. E. *J. Med. Chem*. **1978**, *21*, 1007–1011.

[15] Srinivas, B.; Kumar, V. P.; Sridhar, R.; Prakash Reddy, V.; Nageswar, Y. V. D.; Rao, K. R. *Helv. Chim. Acta* **2009**, *92*, 1080–1084.

[16] For examples of abnormal ozonolysis in steroid series, see: (a) Li, Z.; Heffner, P.; Gong, Y. *J. Label. Compd. Radiopharm.* **2001**, *54*, 839–846. (b) Cabaj, J. E.; Kairys, D. ; Benson, T. R. *Org. Process Res. Dev.* **2007**, *11*, 378–388.

[17] The term abnormal ozonolysis refers to a transformation with ozone in which not only the C-C double bond but also one of the adjacent C-C single bonds is cleaved: α,β-unsaturated carbonyl compounds are typical substrates for such a process.

[18] For some investigations concerning abnormal ozonolysis mechanisms, see: (a) Dauben, W.; Wight, H.; Boswell, G. *J. Org. Chem*. **1958**, 23 (11), 1787–1788. (b) Yamamoto, Y.; Niki, E.; Kamiya, Y. *J. Org. Chem.* **1981,** *46*, 250–254. (c) Barron, E.; Deborde, M.; Rabouan, S.; Mazellier, P.; Legube, B. *Water Res.*

2006, *40*, 2181–2189. (d) Zvereva, T. I.; Kasradze, V. G.; Kazakova, O. B.; Kukovinets, O. S. *Russ. J. Org. Chem.* **2010,** *46*, 1431–1451.

[19] Borthakur, M.; Boruah, R. C. *Steroids* **2008**, *73*, 637-641.

[20] (a) Liu, Y.; Kim, K. E.; Herberta, M. B.; Fedorov, A.; Grubbs, R. H.; Stoltz, B. M. *Adv. Synth. Catal*. **2014**, *356*, 130–136. (b) Chatterjee, A.; Eliasson, S. H. H.; Törnroos, K. W.; Jensen, V. R. *ACS Catal*. **2016**, *6*, 7784–7789.(c) Chatterjee, A.; Anamitra, J.; Vidar, R. *ACS Catal.* **2017**, *7*, 2543–2547.

[21] For a recent example, see: Liu, Y.; Virgil, S. C.; Grubbs, R. H.; Stoltz, B. M. *Angew. Chem. Int. Ed*. **2015**, *54*, 11800–11803.

[22] Le Nôtre, J.; Scott, E. L.; Franssen, M. C. R.; Sanders, J. P. M. *Tetrahedron Letters* **2010**, *51*, 3712–3715.

[23] Miller, J. A.; Nelson, J. A.; Byrne, M. P. *J. Org. Chem*. **1993**, *58*, 18–20.

[24] Zhong, Y.-L.; Shing, T. K. M. *J. Org. Chem*. **1997**, *62*, 2622–2624.

[25] For preparation of [1,2,3-^{13}C3]-1-(triphenylphosphoranylidene)-2-propanone **2**, see reference 12a.

[26] Spinella, A.; Fortunati, T.; Soriente, A. *Synlett* **1997**, 93–94.

[27] Rüchardt, C.; Eichler, S.; Panse, P. *Angew. Chem. Int. Ed*. **1963**, *2*, 619.

[28] Patil, V. J.; Mävers, U. *Tetrahedron Lett.* **1996**, *37*, 1281–1284.

[29] Dambacher, J.; Zhao, W.; El-Batta, A.; Anness, R.; Jiang, C.; Bergdahl, M. *Tetrahedron Lett.* **2005**, *46*, 4473–4477.

[30] (a) Ihara, M.; Tokunaga, Y.; Taniguchi, N.; Fukumoto K. *Tetrahedron*, **1991**, *47*, 6635–6648. (b) Nakajima, T.; Yamashita, D.; Suzuki, K.; Nakazaki, A.; Suzuki, T.; Kobayashi, S. *Org. Lett.* **2001**, *13*, 2980–2983. (c) Kim, P.; Barry, C. E.; Dowd, C. S. *Tetrahedron Lett.* **2006**, *47*, 3447–3451.

[31] Thangaraj, K.; Srinivasan, P. C.; Swaminathan, S. *Synthesis* **1982,** *10*, 855–857.

[32] (a) Tweit, R. C. *J. Org. Chem*. **1962**, *27*, 2693–2694. (b) Dodson, R. M.; Tweit, R. C. *J. Am. Chem. Soc.* **1959**, *81*, 1224–1227.

[33] Yang, Y.; Haino, T.; Usiu, S.; Fukazawa; Y. *Tetrahedron* **1996**, *52*, 2325–2336.

[34] Claramunt, R. M.; Sanz, D.; Alkorta, I.; Elguero, J.; Foces-Foces, C.; Llamas-Saiz, A. L. *J. Heterocyclic Chem*. **2001**, *38*, 443-449.

[35] Braddock, D. C.; Clarke, J.; Rzepa, H. S. *Chem. Commun*. **2013**, *49*, 11176-11178.

[36] Wube, A. A.; Hüfner, A.; Thomaschitz, C.; Blunder, M.; Kollroser, M.; Bauer, R.; Bucar, F. *Bioorganic. Med. Chem.* **2011**, *19*, 567-579.

[37] Lin, Y.; Song, X.; Fu, J.; Lin J.; Qu, Y. *J. Chem. Technol. Biotechnol.* **2009**, *84*, 789–793.