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A safe and practical method for the preparation of 7α-thioether and thioester derivatives of spironolactone

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ABSTRACT

Spironolactone is a renal competitive aldosterone antagonist. One of its most important metabolite is the 7α-methylthio spironolactone: thus it is very important to have an efficient and safe access to this compound, for pharmacokinetic studies. In this context, we synthesized this metabolite by thioalkylation of 7α-thio spironolactone using Hünig's base with a very good yield. We also used our procedure to prepare, with an easy work-up and high yields, 7α-thioether and thioester derivatives of spironolactone, that could be useful for further Structure–Activity Relationships studies.

1. Introduction

Spironolactone (SL, **1**), a C19-steroidal compound, having a spirannic γ-lactone at the C-17 position is a well-known anti-aldosterone [1,2]. SL has been widely used for over 40 years to treat fluid retention, mild high blood pressure and a few rare hormonal problems [3–5]. *In vivo* studies have shown that the two major hepatic metabolites of SL are canrenone (CAN, **2**) and 7α-methylthio SL (TM, **3**) [6,7] (Fig. 1). Both metabolites contribute to the antimineralocorticoid effects of SL; however, the higher affinity for renal aldosterone receptors combined with the higher plasma concentrations of TM, indicate that this metabolite plays a major role for these effects [8,9]. Consequently, there is a need for pharmacokinetic studies on SL to have this metabolite in reasonable quantities: this prompted us to develop an easy preparation of 7α-thiomethylspironolactone **3** and the first part of this work will describe our optimization process, implying the methylation of a 7α-thiol precursor.

Furthermore, a recent review [10] reported an additional biological activity of SL as an inhibitor of type 2 17β-hydroxysteroid dehydrogenase (17β-HSD). Investigations on spiro-γ-lactones demonstrated the biological interest of C18-steroidal lactones [11] and some SL analogues [12]. Particularly, the 7α-thioacetyl group was modified with various thioalkyl and thioester side chains to enhance the inhibitory potency of SL. So, the second aim of the methodology we described herein was to bring an easy access to such derivatives.

2. Experimental

2.1. General remarks

Spironolactone was purchased from Amplachem, USA. 4-Methoxybenzyl bromide (**5a**) [13] and 4-*t*-butyldimethylsilyloxybenzyl bromide (**5b**) [14] were prepared as described in the literature. All reagents and solvents were purchased from Acros Organics or Sigma Aldrich and were used without further purification. Reactions were monitored by thin layer chromatography (TLC, UV_{254nm}) aluminum sheets coated with Kieselgel $60F_{254}$. Product purification by flash column chromatography was performed using Kieselgel 60 Å (40–63 μm). Melting points were determined on a differential scanning calorimeter apparatus, Q200® from TA Instruments. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Brüker ALS300 and DRX300 Fourier transform spectrometers, using an internal deuterium lock, operating at 300 MHz. Chemical shifts are reported in parts per million (ppm) relative to internal standard (tetramethylsilane, TMS). Data are presented as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet), coupling

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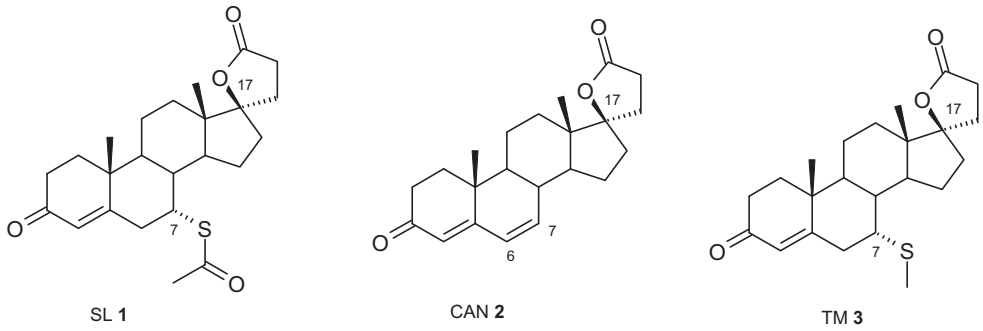


Fig. 1. Chemical structures of SL **1** and its two metabolites, CAN **2** and TM **3**.

constant (*J*, reported in Hz), integration, assignment. Carbon magnetic resonance (^{13}C NMR) spectra were recorded on Brüker DRX300 and DRX400 Fourier transform spectrometers, using an internal deuterium lock, operating at 75 and 100 MHz, respectively. FT-IR spectra were recorded on IR Prestige-21 from Shimadzu. Electrospray ionization (ESI) mass spectra were recorded on an Agilent 1290 system coupled with 6120 single quadrupole Mass Spectrometer. High-resolution mass spectra were recorded on a Brüker MicroTOF Q Mass Spectrometer.

*2.2. Synthesis of 3-oxo-17α-pregna-4-ene-7α-(thia)-21,17-carbolactone (**4**)*

Sodium methoxide was first prepared by adding sodium (1.3 g, 56 mmol) to cold methanol (0 °C) (96 mL). SL **1**, (10.0 g, 24 mmol) was then added to this solution and the resulting mixture was stirred at room temperature for 40 min. Then, the reaction mixture was neutralized (pH 7) with acetic acid and water (200 mL) was added. The beige solid (13.6 g) was recovered by filtration and recrystallized with methanol (130 mL) to afford compound **4** (6.0 g, 69%) as a yellow powder. The physical and spectral data were identical to those reported in the literature [15]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (s, 3H, 18-CH_3), 1.20 (s, 3H, 19-CH_3), 3.33–3.27 (m, 1H, 7β-CH), 5.79 (s, 1H, 4-CH).

*2.3. General procedure for the synthesis of compounds **3** and **7a–h***

To a solution of diisopropylethylamine (DIPEA) (0.44 mL, 2.5 mmol) in acetonitrile (7 mL) was added compound **4** (374 mg, 1 mmol) followed by the corresponding halide (1.2 mmol). At the end of the reaction, the volatiles were removed under reduced pressure and the crude product was then purified by column chromatography.

*2.3.1. 3-Oxo-17α-pregna-4-ene-7α-(methylthia)-21,17-carbolactone (TM **3**)*

According to general procedure, scale: CH_3CN (7 mL), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), methyliodide (0.074 mL, 1.2 mmol); reaction time at rt = 30 min. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound TM **3** (300 mg, 77%) as a white solid; the spectral data were identical to those reported in the literature [15]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (s, 3H, 18-CH_3), 1.21 (s, 3H, 19-CH_3), 2.04 (s, 3H, S-CH_3), 2.95–2.98 (m, 1H, 7β-CH), 5.78 (s, 1H, 4-CH).

*2.3.2. 3-Oxo-17α-pregna-4-ene-7α-(butylthia)-21,17-carbolactone (**7a**)*

According to general procedure, scale: CH_3CN (7 mL), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), butyl bromide (0.34 mL, 1.2 mmol); reaction time at 80 °C = 24 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7a** (183 mg, 43%) as a white solid; the spectral data were identical to those reported in the literature [16]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J = 7.2, 3H, S-$(CH_2)_3$–CH_3), 0.97 (s, 3H, 18-CH_3), 1.20 (s, 3H, 19-CH_3), 3.02–3.05 (m, 1H, 7β-CH), 5.77 (s, 1H, 4-CH).

*2.3.3. 3-Oxo-17α-pregna-4-ene-7α-(methylacetylthia)-21,17-carbolactone (**7b**)*

According to general procedure, scale: CH_3CN (7 mL), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), methyl α-bromoacetate (0.11 mL, 1.2 mmol); reaction time at 80 °C = 1 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7b** (390 mg, 87%) as a white solid; mp 182–185 °C. IR ν_{max} (cm^{-1}): 1768, 1730, 1650, 1616. ^{1}H ($CDCl_3$, 300 MHz): δ 0.97(s, 3H, 18-CH_3), 1.22 (s, 3H, 19-CH_3), 3.13–3.26 (m, 3H, CH_2–CO and 7β-CH), 3.73 (s, 3H, O–CH_3), 5.76 (s, 1H, 4-CH). ^{13}C ($CDCl_3$, 300 MHz): δ 198.2 (C-3), 176.4 (C-22), 170.3 (C-2′), 166.0 (C-5), 158.7 (C-5′), 126.7 (C-4), 95.4 (C-17), 52.2 (C-3′), 46.7 (C-9), 45.6 (C-13), 45.1 (C-14), 44.0 (C-7), 39.4 (C-8), 38.1 (C-10), 37.3 (C-6), 35.2 (C-1), 34.9 (C-16), 33.6 (C-1′), 31.4 (C-2), 30.9 (C-20), 30.8 (C-21), 28.9 (C-2), 21.7 (C-11), 20.2 (C-15), 17.6 (C-19), 14.2 (C-18). HRMS (ESI+) *m*/*z* calcd for $C_{25}H_{34}NaO_2S$ $(M+Na)^+$, 469.2006; found, 469.2019.

*2.3.4. 3-Oxo-17α-pregna-4-ene-7α-(allylthia)-21,17-carbolactone (**7c**)*

According to general procedure, scale: CH_3CN (7 mL), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), allyl bromide (0.10 mL, 1.2 mmol); reaction time at 80 °C = 2 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7c** (318 mg, 77%) as a white solid; the spectral data were identical to those reported in the literature [16]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (s, 3H, 18-CH_3), 1.21 (s, 3H, 19-CH_3), 2.98–3.02 (m, 1H, 7β-CH), 3.06–3.11 (m, 2H, CH_2=CH–CH_2), 5.05–5.11 (m, 2H, CH_2=CH–CH_2), 5.67–5.80 (m, 2H, 4-CH and CH_2=CH–CH_2).

*2.3.5. 3-Oxo-17α-pregna-4-ene-7α-(propargylthia)-21,17-carbolactone (**7d**)*

According to general procedure, scale: CH_3CN (7 mL), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), propargyl bromide (0.10 mL, 1.2 mmol); reaction time at 80 °C = 1 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7d** (360 mg, 79%) as a white solid; mp 172 °C (dec). IR ν_{max} (cm^{-1}): 3223, 1762, 1649, 1618, 1170. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (s, 3H, 18-CH_3), 1.22 (s, 3H, 19-CH_3), 3.20 (dd, J = 17.1–2.5, 1H, CH_2–C≡CH), 3.27 (dd, J = 17.1–2.5, 1H, CH_2–C≡CH) 3.34–3.37 (m, 1H, 7β-CH), 5.77 (d, J = 1.2, 1H, 4-CH). ^{13}C ($CDCl_3$, 300 MHz): δ 198.4 (C-3), 176.5 (C-

22), 166.3 (C-5), 126.8 (C-4), 95.5 (C-17), 79.4 (C-2′), 71.2 (C-3′), 47.3 (C-9), 45.2 (C-13), 45.1 (C14), 45.0 (C-7), 39.5 (C-8), 38.2 (C-10), 37.5 (C-6), 35.3 (C-1), 35.0 (C-16), 33.7 (C-1′), 31.0 (C-20), 31.0 (C-21), 29.0 (C-2 et C-12), 21.8 (C-11), 20.3 (C-15), 17.7 (C-19), 14.3 (C-18). HRMS (ESI+) *m/z* calcd for $C_{25}H_{32}NaO_3S$ $(M+Na)^+$, 435.1955; found, 435.1964.

*2.3.6. 3-Oxo-17α-pregna-4-ene-7α-(benzylthia)-21,17-carbolactone (**7e**)*

According to general procedure, scale: CH_3CN (7 mL), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), benzyl bromide (0.14 mL, 1.2 mmol); reaction time at 80 °C = 2 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7e** (326 mg, 77%) as a white solid; the spectral data were identical to those reported in the literature [12]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (s, 3H, 18-CH_3), 1.11 (s, 3H, 19-CH_3), 2.79–2.82 (m, 1H, 7β-CH), 3.51 (q, J = 13.5, 2H, CH_2-Ph), 5.67 (d, J = 1.5, 1H, 4-CH), 7.14–7.20 (m, 5H, Ph).

*2.3.7. 3-Oxo-17α-pregna-4-ene-7α-(p-methoxybenzylthia)-21,17-carbolactone (**7f**)*

According to general procedure, scale: CH_3CN (7 ml), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), 4-Methoxybenzyl bromide (**5a**) (241 mg, 1.2 mmol); reaction time at 80 °C = 4 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7f** (350 mg, 71%) as a white solid; mp 228–233 °C. IR ν_{max} (cm^{-1}):1762, 1734, 1665, 1608, 1170. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (s, 3H, 18-CH_3), 1.18 (s, 3H, 19-CH_3), 2.85–2.87 (m, 1H, 7β-CH), 3.61 (q, J = 12, 2H, CH_2-Ph), 3.78 (s, 3H, O–CH_3), 5.74 (s, 1H, 4-CH), 6.82 (d, J = 9, 2H, C_6H_5), 7.18 (d, J = 9, 9H, C_6H_5). ^{13}C ($CDCl_3$, 400 MHz): δ 198.5 (C-3), 176.6 (C-22), 166.9 (C-5), 158.6 (C-5′), 129.8 (C-3′), 129.7 (C-2′), 126.7 (C-4), 113.7 (C-4′), 95.6 (C-17), 55.1 (C-6′), 47.1 (C-9), 45.1 (C-13), 44.9 (C-14), 44.5 (C-7), 39.6 (C-8), 38.2 (C-10), 38.0 (C-6), 35.3 (C-1), 35.1 (C-16), 34.3 (C-1′), 33.8 (C-2), 31.1 (C-12), 31.1 (C-20), 29.1 (C-21), 21.7 (C-11), 20.3 (C-15), 17.7 (C-19), 14.3 (C-18). $C_{30}H_{38}NaO_4S$ $(M+Na)^+$, 517.2385; found, 517.2383.

*2.3.8. 3-Oxo-17α-pregna-4-ene-7α-[(4-t-butyldimethylsilyloxy)-benzylthia)]-21,17-carbolactone (**7g**)*

According to general procedure, scale: CH_3CN (3.5 ml), DIPEA (0.22 mL, 1.75 mmol), compound **4** (187 mg, 0.5 mmol), 4-*t*-butyldimethylsilyloxybenzyl bromide (**5b**) (180.6 mg, 0.6 mmol); reaction time at 60 °C = 2 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7g** (210 mg, 71%) as a white solid; the spectral data were identical to those reported in the literature [12]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.17 (s, 6H, 2 Si–(CH_3)), 0.92 (s, 3H, 18-CH_3), 0.97 (s, 9H, Si–C–(CH_3)$_3$), 1.118 (s, 3H, 19-CH_3), 2.83–2.85 (m, 1H, 7β-CH), 3.59 (q, J = 15.0, 2H, CH_2Ph), 5.75 (s, 1H, 4-CH), 6.76 (d, J = 9, 2H, Ph), 7.12 (d, J = 8.4, 2H, Ph).

*2.3.9. 3-Oxo-17α-pregna-4-ene-7α-(benzoylthia)-21,17-carbolactone (**7h**)*

According to general procedure, scale: CH_3CN (7 mL), DIPEA (0.44 mL, 2.5 mmol), compound **4** (374 mg, 1 mmol), benzoyl chloride (0.14 mL, 1.2 mmol); reaction time at 80 °C = 1 h. The crude product was purified by flash chromatography (cyclohexane/EtOAc 7/3) to afford compound **7h** (290 mg, 75%) as a yellow solid; the spectral data were identical to those reported in the literature [12]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (s, 3H, 18-CH_3), 1.27 (s, 3H, 19-CH_3), 4.23–4.26 (m, 1H, 7β-CH), 5.71 (s, 1H, 4-CH), 7.45 (t, J = 7.8, 2H, Ph), 7.59 (t, J = 7.5, 1H, Ph), 7.96 (d, J = 7.2, 2H, Ph).

3. Results and discussion

The first part of this work was to find an efficient synthesis of metabolite TM **3**. In the 70's Karim and Brown [15] proposed the preparation of this compound using a conjugated addition of gaseous methyl mercaptan on CAN **2**.

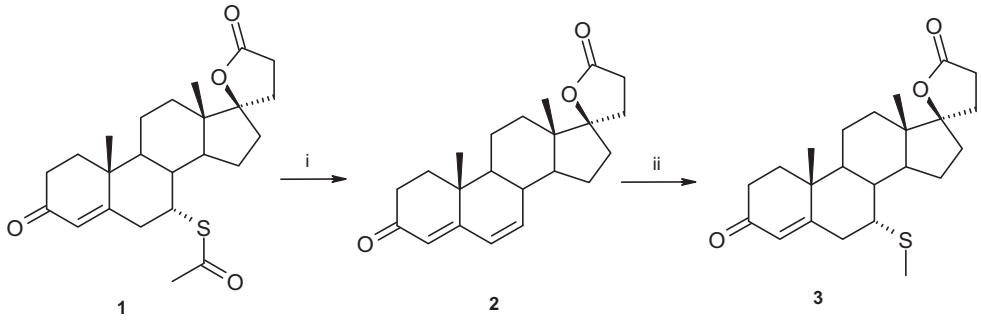
CAN **2** was first prepared in a good yield from SL **1**, after removal under basic conditions (excess of MeONa) of the thioacetyl group, according to a retro-Michael elimination (Scheme 1) [12]. In a second step, a conjugated 1,6-addition of gaseous methylmercaptan under pressure, led to the desired 7α-thiomethyl derivative (TM **3**) after 20 h of reaction time. Beside the major drawback of this method, i.e. the use of a toxic gas in drastic reaction conditions (and the olfactive nuisance as well), there was a lack of versatility for this approach, which also requires a long reaction time for the functionalization second step.

In order to improve this synthesis, we decided to design a new two steps pathway, based on an alkylation reaction of a 7α-thiol derivative **4** (Scheme 2), thus avoiding the use of gaseous MeSH.

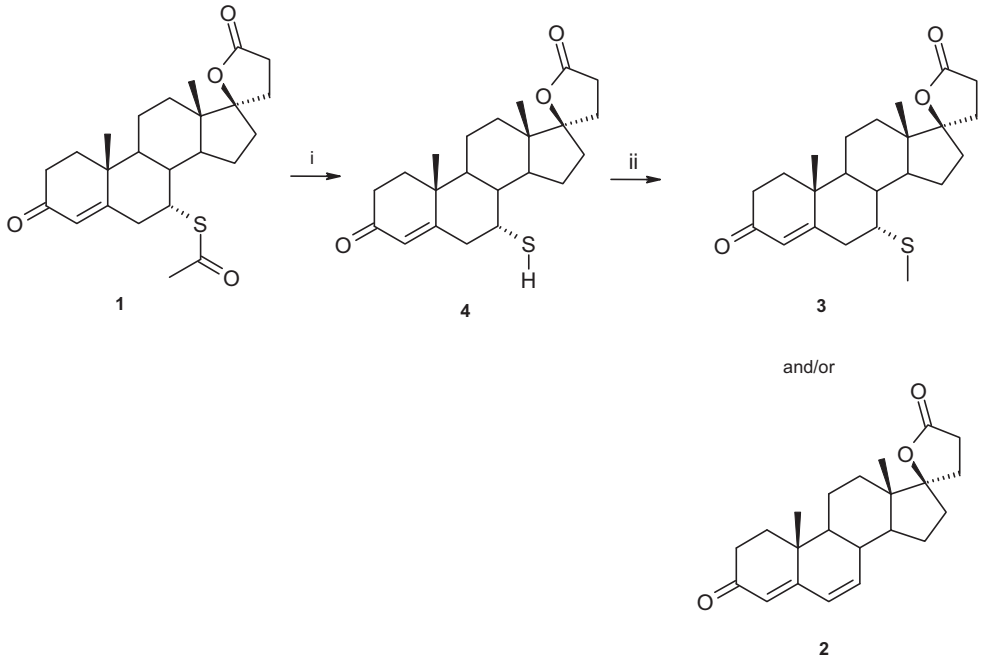
First, the acetyl group of SL **1** was cleaved using sodium methoxide in methanol to yield 7α thiol derivative **4** (Scheme 2) [17].

In the second key step, methyl iodide was used as the electrophile for the alkylation of the thiol function of **4** and, to determine the optimal conditions, both the nature of base and solvent were investigated (Table 1).

Initially, the reaction was carried out using a slight excess of sodium methoxide in methanol [18] but, unfortunately, the expected product TM **3** was not observed as CAN **2** was detected as the sole product (Table 1, entry 1), isolated with a 14% yield. The use of sodium hydride as the base improved the reaction as compound TM **3** was detected in the crude product (Table 1, entry 2): CAN **2** was still obtained as the major product. The formation of this undesired compound seemed to occur when the concentration of the thiolate anion was high, compared to the quantity of the MeI electrophile:



Scheme 1. Reagents and conditions: (i) MeONa 5.8 equiv, THF, rt, 18 h; (ii) piperidine, gaseous MeSH, MeOH, rt, 20 h.



Scheme 2. Reagents and conditions: (i) MeONa 2.8 equiv, MeOH, rt, 40 min, 69%; (ii) MeI 1.2 equiv, base, solvent.

Table 1
Optimization of reaction conditions for preparation of TM **3**.

Entry[a]	Base	Equivalent	Solvent	Time (h)	Ratio **2**/**3**/**4**[b]	Yield of TM **3** (%)
1	MeONa	1.2	MeOH	1	100/0/0	0
2	NaH	1.2	THF	2	71/29/0[c]	nd
3	NaH[d]	1.2	THF	2	2/98/0	67
4	NEt_3	2.5	THF	2	1/33/66	nd
5	DIPEA	2.5	THF	2	1/99/0	71
6	DIPEA	2.5	CH_3CN	0.5	1/99/0	77

nd, not determined.
[a] All reactions were performed at room temperature and MeI was added at the end, except for entry 3.
[b] Determined by ^1H NMR analysis of the crude product, except for entry 2.
[c] Determined by LC/MS analysis of the crude product.
[d] *In situ* quench: the electrophile MeI was added before the base.

the high concentration of this basic species might probably be at the origin of the elimination process leading to CAN **2**.

To confirm this hypothesis, we then decided to carry out an *in situ* trapping of the anion with the electrophile and this was realized by doing a "reverse addition" of the electrophile. These conditions have drastically limited CAN **2** formation and compound TM **3** was obtained with a 67% yield (Table 1, entry 3).

The use of an organic base, such as triethylamine, to trap the hydroiodic acid released after the alkylation was envisaged and this modification reduced CAN **2** formation, but also reaction rate (Table 1, entry 4), as starting material **4** was detected after a 2 h reaction time. We then turned our attention to a more basic tertiary amine, diisopropylethylamine (DIPEA), also known as Hünig's base, to promote the reaction: in this case, only trace amount of the side-product CAN **2** was detected and the desired compound TM **3** was obtained with a good 71% isolated yield (Table 1, entry 5).

The use of acetonitrile instead of THF dramatically reduced the reaction time, from 2 h to 30 min, and slightly improved the yield (Table 1, entry 6), which constitutes an ultimate improvement for this reaction.

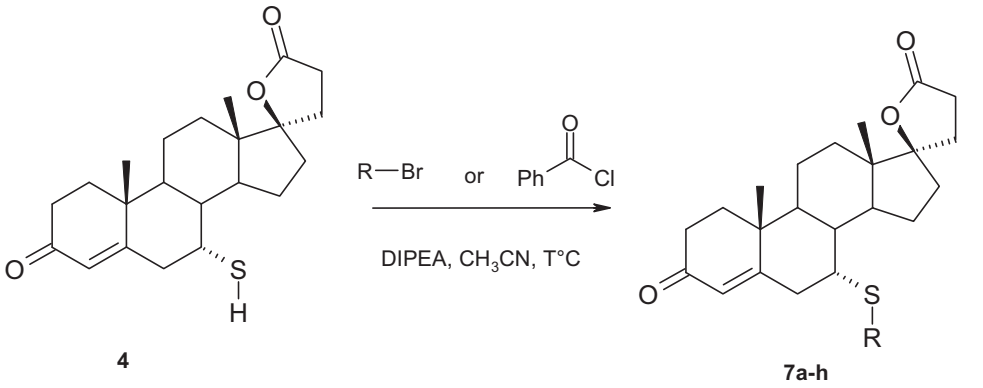
With these optimal conditions in hand, we then decided to study the scope of this reaction by synthesizing several 7α-thioalkyl SL derivatives (Scheme 3 and Table 2).

Compound **7a** was synthesized using bromobutane which was much less reactive than iodomethane, thus leading to the expected product in a low yield, though after a very long reaction time (Table 2, entry 1).

Yield substantially increased when methyl bromoacetate was used for the preparation of compound **7b**, which was obtained with an excellent 87% isolated yield (Table 2, entry 2).

It is also important to point out that our protocol is very simple and convenient, first for the synthesis, but also for the work-up: after evaporation of acetonitrile, the crude product was directly purified by flash chromatography. This new two-step method for the preparation of diverse derivatives of SL has been guided by the principle of "benign by design" [19]. The process avoids both the use of gaseous and malodorous reagents and the use of additional solvents for a supplementary extraction step.

The use of activated α-unsaturated halides such as allyl and propargyl bromide, provided compounds **7c** and **7d** with good 77% and 79% yields, respectively (Table 2, entries 3 and 4). These derivatives **7c** and **7d** could be used as precursors for a subsequent grafting of functional groups on the 7α side-chain, by employing Heck or cross-metathesis reactions on the double unsaturation or Sonogashira coupling reaction on the triple bond.



7a (R = *n*-Bu)
7b (R = CH_2CO_2Me
7c (R = CH_2-CH=CH_2)
7d (R = CH_2-CCH)
7e (R = Bn)
7f (R = CH_2-Ph-*p*-OMe)
7g (R = CH_2-Ph-*p*-OTBDMS)
7h (R = COPh)

Scheme 3. The reaction was carried out with the conditions obtained for the synthesis of compound TM **3** but, as the alkyl bromides we used were less reactive than methyl iodide, the temperature was increased to 60–80 °C in order to reduce the reaction time (Table 2).

Table 2
Thioalkylation of **4** in the presence of DIPEA (2.5 equiv) in CH_3CN.

Entry	Compound	R	Reaction conditions (time, temp.)	Yield (%)
1	**7a**	*n*-Bu	24 h, 80 °C	43
2	**7b**	CH_2–CO_2Me	1 h, 80 °C	87
3	**7c**	CH_2–CH=CH_2	2 h, 80 °C	77
4	**7d**	CH_2–C≡CH	1 h, 80 °C	79
5	**7e**	Bn	2 h, 80 °C	77
6	**7f**	CH_2-Ph-*p*-OMe	4 h, 80 °C	71
7	**7g**	CH_2-Ph-*p*-OTBDMS	2 h, 60 °C	71
8	**7h**	COPh[a]	1 h, 80 °C	75

[a] Benzoyl chloride was used.

According to our methodology, compounds **7e**–**g** were synthesized in good yields (Table 2, entries 5–7) using the corresponding benzyl bromides [20]. **7f** and **7g** are the precursors of the phenolic derivative, a known inhibitor of type 2 17β-HSD [12], and this function could be used to attach linkers (e.g. polyethyleneglycol, frequently reported in the literature [21] as biocompatible and "easily-tunable", for its length) on the steroid.

Finally, our approach is also applicable to benzoyl chlorides, as shown by the preparation of 7α-thiobenzoate derivative **7h** (Table 2, entry 8): this last example illustrates the fact that our methodology is also applicable for the preparation of various thioester derivatives of spironolactone.

In conclusion, a new method for the preparation of 7α-thiomethyl metabolite of spironolactone **3** was developed. Our protocol used safe conditions and easy work-up, gave very good yield, with one exception for alkyl bromides, and proved to be versatile. Our approach can indeed be applied for the preparation of new steroidal derivatives that could be useful for further functionalization and so to study new Structure–Activity Relationships.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2012.09.005.

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