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Novel pathway for the synthesis of arylpropionamide-derived selective androgen receptor modulator (SARM) metabolites of andarine and ostarine

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ABSTRACT

O-Dephenylandarine and *O*-dephenylostarine, two SARM metabolites relevant for doping control analysis, were synthesized in their endogenous (*S*)-forms as well as in terms of their racemates. The enantiopure (*S*)-metabolites were obtained after six steps in 20% and 23% overall yield, the slightly modified racemic route provided the compounds in 28% and 31% total yield, respectively.

Selective androgen receptor modulators (SARMs) have been intensively investigated during the past decade, because of their potential ability to replace anabolic–androgenic steroids in the treatment of hypogonadism, muscle wasting, osteoporosis, or benign prostatic hyperplasia. By showing the same effectiveness in activating the androgen receptor, nonsteroidal SARMs seem to be more tolerable concerning undesirable side effects as a result of their markedly high selectivity to muscle and bone tissue.[1–6]

These promising first results inevitably also attracted athletes, seeking enhanced physical performance and strength, willing to achieve their goals even illegally by the misuse of drugs. Although by now none of the lead candidates has received clinical approval, the availability of andarine (S-4) (**2a**) via the internet as well as its actual in-competition administration, resulting in an adverse analytical finding, has been reported.[7–10] Accordingly, the whole class of SARMs has been included in the list of prohibited substances by the World Anti-Doping Agency (WADA) since 2008.

Andarine and structurally closely related ostarine (also referred to as S-22) (**2b**) belong to the class of arylpropionamide-based SARMs, which are structurally derived from the anti-androgen bicalutamide (**1**) (Scheme 1). The latter was first synthesized by Tucker et al.[11–15] For the detection of SARMs for doping control purposes, reference material in terms of the most abundant urinary metabolites is needed. Possible targets for doping analysis were revealed by a series of metabolism studies including in vitro and animal/human in vivo data.[16–22] Besides other phase-I modifications both drug candidates undergo hydrolysis of the ether linkage resulting in the dephenylated molecules **3a** and **3b**. Due to phase-II glucuronidation also the corresponding conjugates are detected in human urine specimens after oral administration of SARMs S-4 and S-22, respectively.[21,22] Accordingly these glucuronides have to be hydrolyzed prior to analysis. In 2008 Thevis and coworkers synthesized andarine's major metabolite *O*-dephenylandarine (**3a**).[23]

Herein, we want to present a new, simple route for the efficient preparation of *O*-dephenylandarine (**3a**) and *O*-dephenylostarine (**3b**). These metabolic products of **2a** and **2b** represent valuable reference substances, allowing the unequivocal proof of illicit SARM administration by athletes (Scheme 1).

Based on the fact, that reference substances for routine doping control purposes do not have to fulfill the criterion of enantiopurity, we first concentrated on the preparation of racemic **3a** and **3b**. We succeeded in creating a short, highly cost-effective way, which provides *rac*-**3a** in 28% and *rac*-**3b** in 31% yield over five reaction steps (Scheme 2). The synthesis of racemic metabolites starts with the epoxidation of methacrylic acid **4**, with mCPBA[24] to give *rac*-**5** in 70% yield. The following formation of the amide bond between *rac*-**5** and anilines **6** represents the key step in the formation of the arylpropionamide core structure. Our first attempts to achieve this via activation of epoxy acid with isobutyl chloroformate,[25] Yamaguchi reagent (2,4,6-trichlorobenzoyl chloride),[26] or with TBTU[27] failed and resulted in a total loss of the starting material. Most probably the lack of reaction is due to the poor

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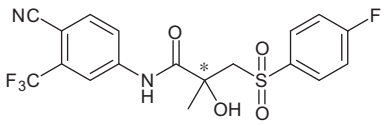
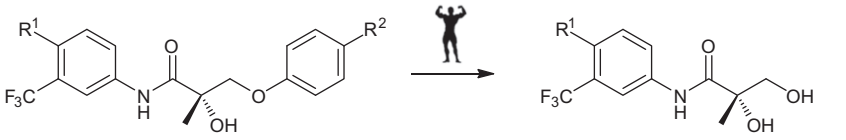


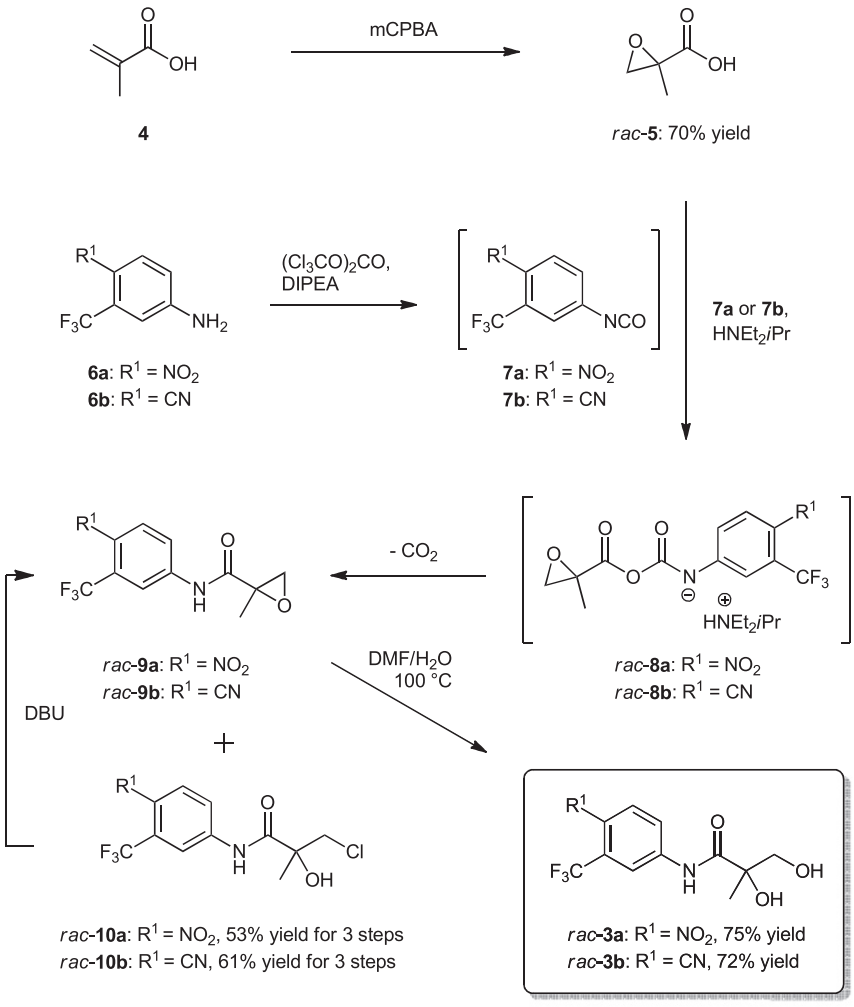
bicalutamide (Casodex) (**1**)



andarine (**2a**): $R^1 = NO_2$, R^2 = NHAc
ostarine (**2b**): R^1 = CN, R^2 = CN

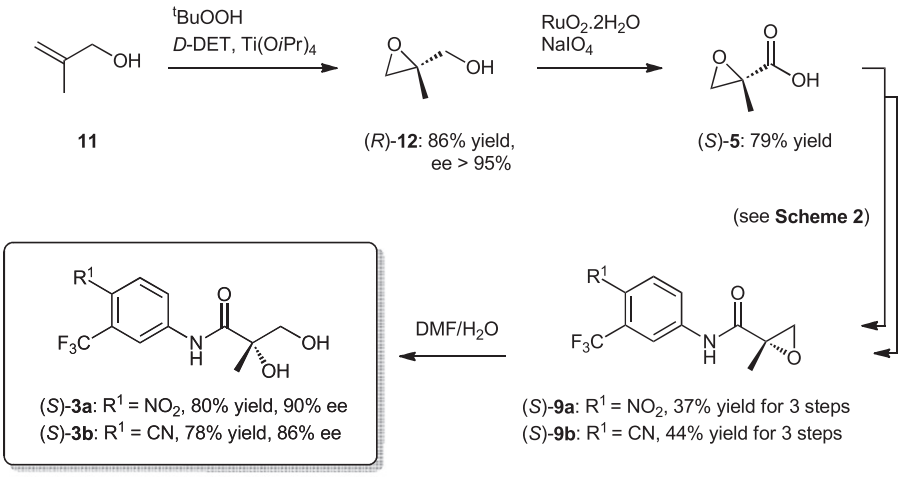
(*S*)-*O*-dephenylandarine (**3a**) : $R^1 = NO_2$
(*S*)-*O*-dephenylostarine (**3b**): R^1 = CN

Scheme 1. Chemical structures of bicalutamide and the urinary metabolites of andarine and ostarine.



Scheme 2. Synthesis of racemic *O*-dephenylandarine and *O*-dephenylostarine.

nucleophilicity of the aromatic amino group, which is additionally deactivated by the electron-withdrawing nitro or cyano substituent. Finally, according to the Crich procedure[28], the DIPEA-salt of epoxy acid *rac*-**5** was reacted with in situ generated isocyanates **7**[29] to give intermediate **8** which after decarboxylation yielded the desired epoxyamide *rac*-**9** together with the corresponding chlorohydrins *rac*-**10**. The formation of the latter could be explained by the presence of chloride ions in the reaction mixture due to the application of triphosgene. Conversion of *rac*-**10** to *rac*-**9** was easily achieved by treatment of the corresponding chlorohydrine with DBU to give *rac*-**9a** and *rac*-**9b**, respectively, in good yields over three steps. For the epoxide opening reaction several



Scheme 3. Synthesis of (*S*)-*O*-dephenylandarine and (*S*)-*O*-dephenylostarine.

conditions were screened. Where the reaction of *rac*-**9** with 2 N NaOH led to amide cleavage, various acidic reagents proved to be efficient. The best results were obtained by refluxing of *rac*-**9** in a DMF/water mixture[30] to give *rac*-*O*-dephenylandarine (**3a**) in 75% yield and *rac*-*O*-dephenylostarine (**3b**) in 72% yield.

Considering the natural occurrence of endogenous metabolites of **2a** and **2b** as enantiopure compounds, we also wanted to provide an enantioselective version of the previously described synthetic pathway. This goal was readily achieved by altering the first reaction step and using allylic alcohol **11** as starting material (Scheme 3). Alcohol **11** was converted into 2-methylglycidol (*R*)-**12** by asymmetric Sharpless epoxidation[31,32] with 86% yield and ee >95%. The enantiomeric excess of the epoxyalcohol (*R*)-**5** was determined by ^{19}F NMR spectroscopy according to the Mosher ester method.[33] Ruthenium-mediated oxidation[25] of (*R*)-**12** gave the corresponding carboxylic acid (*S*)-**5**, which was subjected to amide bond formation according to Crich's procedure as described above. Finally, the epoxide opening in DMF/water-mixture (vide supra) gave (*S*)-*O*-dephenylandarine (**3a**)[34] in 20% overall yield (90% ee)[33] and (*S*)-*O*-dephenylostarine (**3b**)[34] in 23% overall yield (86% ee).[33]

Comparison of these synthetic metabolites with excretion samples, using LC/MS/MS techniques, confirmed their presence in urine specimens after oral administration of SARMs S-4 and S-22, respectively. Hence, we succeeded in creating a convenient synthetic route for the preparation of two SARM metabolites in terms of their racemates, applicable to routine doping control. Slight modifications of this procedure provide access to the enantiopure (*S*)-compounds. Starting from isotopically labeled educts, either the acrylic or the aromatic compounds, the herein presented routes can furthermore be adopted for the preparation of stable labeled versions of **3a** and **3b**. This option is of great interest in the field of doping analysis, because stable isotopically labeled internal standards allow for much easier compound identification.

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Supplementary data

Supplementary data (experimental procedure and spectral data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.02.065.

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34. *Selected data*
Compound (S)-**3a**: mp 119–120 °C. $[\alpha]_D^{20}$ −21.2 (*c* 0.480, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 8.37 (1H, d, *J* = 2.2 Hz, H_{Ar}), 8.14 (1H, dd, *J* = 8.9 and 2.2 Hz, H_{Ar}), 8.03 (1H, d, *J* = 8.9 Hz, H_{Ar}), 3.85 (1H, d, ^2J = 11.1 Hz, HO–CH_2), 3.53 (1H, d, ^2J = 11.1 Hz, HO–CH_2), 1.39 (3H, s, Me). ^{13}C NMR (100 MHz, CD_3OD): δ = 177.0 (q, C=O), 144.2 (q, C_{Ar}), 143.9 (q, C_{Ar}), 127.9 (t, C_{Ar}), 125.3 (q, $^2J_{CF}$ = 33.7 Hz), 124.1 (t, C_{Ar}), 123.5 (q, $^1J_{CF}$ = 271.7 Hz), 119.6 (t, $^3J_{CF}$ = 5.9 Hz), 77.6 (q, C(Me)), 69.4 (t, HO–CH_2), 22.6 (s, Me). IR (neat): 3448 (NH, OH), 3305 (OH), 1686 (CO), 1527 (NO), 1320 (NO), 1141, 1044, 901, 759 cm^{-1}. HRMS (ESI): *m*/*z* $[M-H]^-$ calcd for $C_{11}H_{11}F_3N_2O_5$: 307.0547; found: 307.0554.
Compound (S)-**3b**: mp 113–115 °C. $[\alpha]_D^{20}$ −28.1 (*c* 0.585, MeOH) [lit. [35] $[\alpha]_D^{21}$ −42.2 (*c* 0.945, MeOH), 100% ee]. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.37 (1H, s, H_{Ar}), 8.10 (1H, d, *J* = 8.5 Hz, H_{Ar}), 7.91 (1H, d, *J* = 8.5 Hz, H_{Ar}), 3.85 (1H, d, ^2J = 11.1 Hz, HO–CH_2), 3.52 (1H, d, ^2J = 11.1 Hz, HO–CH_2), 1.38 (3H, s, Me). ^{13}C NMR (100 MHz, CD_3OD): δ = 177.0 (q, C=O), 144.1 (q, C_{Ar}), 137.1 (t, C_{Ar}), 134.2 (q, $^2J_{CF}$ = 32.4 Hz, C_{Ar}), 123.9 (q, $^1J_{CF}$ = 272.9 Hz), 123.7 (t, C_{Ar}), 118.7 (t, $^3J_{CF}$ = 5.1 Hz), 116.7 (q, CN), 104.6 (q, C_{Ar}), 77.6 (q, C(Me)), 69.4 (t, HO–CH_2), 22.6 (s, Me). IR (neat): 3447 (NH, OH), 3298 (OH), 2226 (CN), 1688 (CO), 1504, 1325, 1124, 1049, 620 cm^{-1}. HRMS (ESI): *m*/*z* $[M-H]^-$ calcd for $C_{12}H_{11}F_3N_2O_3$: 287.0649; found: 287.065.
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