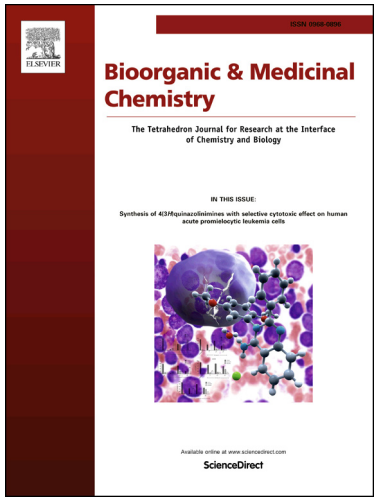
Accepted Manuscript



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Synthesis and biological evaluation of novel Selective Androgen Receptor Modulators (SARMs) Part III: Discovery of 4-(5-oxopyrrolidine-1-yl) benzonitrile derivative 2f as a clinical candidate

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Abstract

We previously reported that 4-(pyrrolidin-1-yl)benzonitrile derivative **1b** was a selective androgen receptor modulator (SARM) that exhibited anabolic effects on organs such as muscles and the central nervous system (CNS), but neutral effects on the prostate. From further modification, we identified that 4-(5-oxopyrrolidine-1-yl)benzonitrile derivative **2a** showed strong AR binding affinity with improved metabolic stabilities. Based on these results, we tried to enhance the AR agonistic activities by modifying the substituents of the 5-oxopyrrolidine ring. As a consequence, we found that 4-[(2*S*,3*S*)-2-ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]-2-(trifluoromethyl)benzonitrile (**2f**) had

ideal SARM profiles in Hershberger assay and sexual behavior induction assay. Furthermore, **2f** showed good pharmacokinetic profiles in rats, dogs, monkeys, excellent nuclear selectivity and acceptable toxicological profiles. We also determined its binding mode by obtaining the co-crystal structures with AR.

Key words: androgen receptor; AR; selective androgen receptor modulators (SARMs); testosterone; Hershberger assay; sexual behavior;

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1. Introduction

Steroid hormones are important chemical messengers which exert various physiological effects.[1–3] Androgen, one of steroid hormones, is a general term of C19 steroid hormones.[4] Androgens increase skeletal muscles, promote osteogenesis, produce erythrocyte and maintain libido through the interaction with androgen receptor (AR). Testosterone is one of the most important androgens which is synthesized mainly from cholesterol in testis and adrenal gland.[5] In the prostate, 5α-reductase converts testosterone to dihydrotestosterone (DHT) with the strongest AR agonistic activity in all naturally occurring androgens.[6,7]

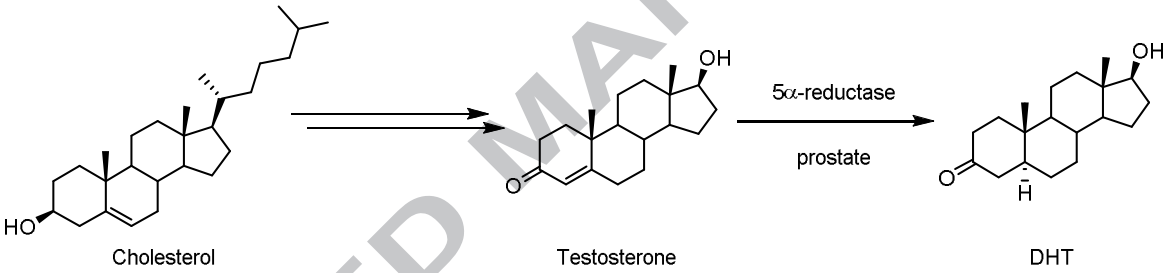


Figure 1. Testosterone Synthesis

In the absence of ligand, AR is maintained in an inactive conformation by HSP70 and HSP 90 in the cytoplasm.[8] The ligand binding causes the C-terminal helix 12 to shift its position to close the ligand binding pocket. The conformational change elicits HSPs' dissociation, leading to homo-dimerization of AR. This facilitates a series of further conformational changes with helix 3 and 5 serving as the key interfaces. The following dissociation of corepressor from the complexes leads to nuclear entry and binding to androgen responsive elements in the promoter of androgen responsive genes. Activated AR has been shown to recruit co-regulators and general transcription factors, which triggers enhancement or repression of target genes transcription.[1,6]

Testicular dysfunction and hypothalamic dysregulation[9] with aging (late on set hypogonadism, LOH)[10] or cachexia which was caused by cancer[11] or COPD,[12] induces a decline in serum testosterone levels, which is believed to be related to various symptoms such as muscle and body weight loss, osteoporosis, depression and decreased libido.[13–15] Several recent clinical studies have reported that androgen replacement therapy (ART) succeeded in increasing lean body mass muscle and decreasing adipose tissue.[16,17] However, use of ART is limited because of the concerns about potential side effects, for example, exacerbation of benign prostatic hypertrophy (BPH) or latent prostate cancer. In addition, testosterone cannot be administered orally due to its rapid hepatic elimination. Consequently, testosterone is administered by less convenient methods, such as intramuscular injection, surgical implantation, or transdermal delivery using patches or gels.[18] Androgenic-anabolic steroids has allowed oral administration, however, hepatotoxicity limits their use in chronic therapy.[19,20]

Under these situations, nonsteroidal and bioavailable tissue selective androgen receptor modulators (SARMs) have received a lot of attentions as new medicines for ART.[11, 21–23] The concept of SARMs was brought from selective estrogen receptor modulators (SERMs),[24] which have been clinically used for over 2 decades to supplement the diminishing circulating estrogens for postmenopausal females. SARMs are expected to show anabolic effects on organs such as muscles and the central nervous system (CNS) without influencing the prostate. Non-steroidal SARMs have potential to be orally available and safer medicines, which provide advantages over conventional ART.

Several non-steroidal SARMs, such as Ostarine (GTx/Merck),[25,26] BMS564929 (Bristol-Myers Squibb),[27] LGD2941 (Ligand),[28] VK–5211 (Viking Therapeutic, structure not disclosed)[29], GSK–2881078[30] and DT-200 (Akashi Therapeutics)[31-33] have been reported to be investigated in clinical studies. Particularly, Ostarine has been proven to increase lean body

mass and improve some muscle functions for cancer cachexia patients in a Phase II study, however, further development appears to have been halted for undisclosed reasons. Ostarine is currently under development as a treatment of AR-positive triple-negative breast cancer.[31]

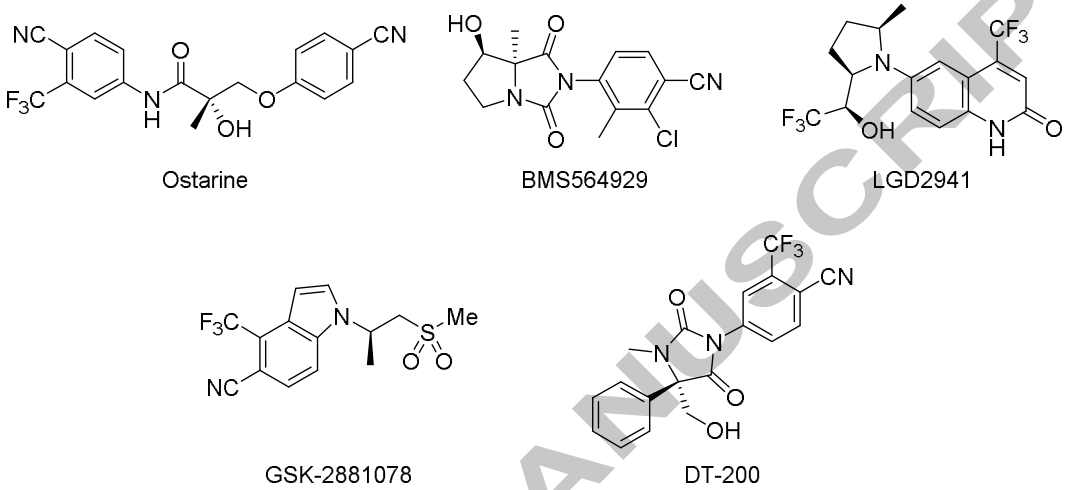


Figure 2. Known nonsteroidal SARMs.

We have previously reported that we found 4-(pyrrolidin-1-yl)benzonitrile derivative **1b** which showed good SARM profiles in vivo, by the modification of substituents on pyrrolidine and benzonitrile of compounds **1a**.[34] In the exploration of this series of compounds, we identified that 4-(5-oxopyrrolidine-1-yl)benzonitrile derivative (**2a**,**b**) showed strong AR binding affinity with improved metabolic stabilities,[35] although their agonistic activities were poor (Figure 3). In order to enhance the agonistic activity of 4-(5-oxopyrrolidine-1-yl)benzonitrile derivatives, we analyzed the co-crystal structure of compound **1a** in detail (Figure 4).[34] The X-ray information suggested that there are two small and hydrophobic pockets around the pyrrolidine ring. One hydrophobic pocket created with Trp741, Met742 and Met745 exists around the 2-position of the pyrrolidine ring of **1a**. On the other hand, the other pocket is constructed by Leu701, Met780, Leu873 and positioned close to the 4-position of the pyrrolidine ring. Furthermore, the SAR study of this series indicated that the introduction of alkyl group at 3-position of the pyrrolidine ring affects not only agonistic

activity but also pharmacokinetic profiles, leading to the discovery of compound **1b**.[34] We hypothesized that the binding mode of **2a** is similar to that of compound **1a**, and designed more potent agonists than compound **2a** by modifying the substituents on the 5-oxopyrrolidine ring (R^1–R^5). In this paper, we will describe the design, synthesis, in vitro, in vivo characterizations of 4-(5-oxopyrrolidine-1-yl)benzonitrile derivatives, and the discovery of 4-[(2*S*,3*S*)-2-ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]-2-(trifluoromethyl)benzonitrile **2f**, which shows ideal SARM profiles.

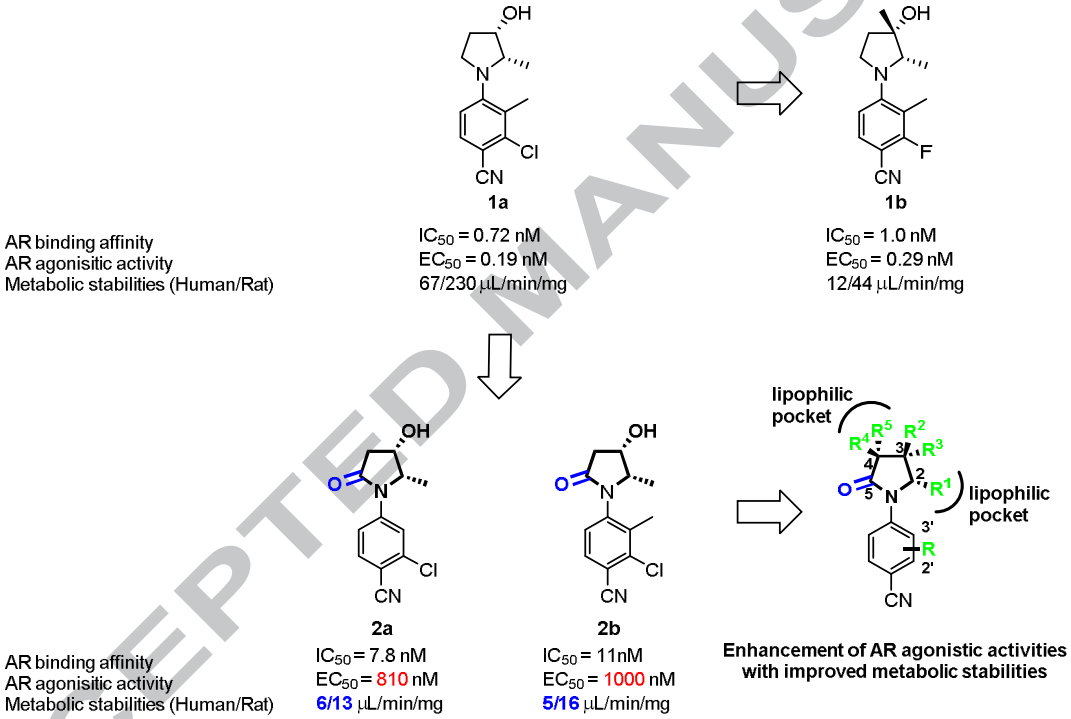


Figure 3. The design of 4-(5-oxopyrrolidine-1-yl)benzonitrile derivatives with more potent AR agonistic activities.

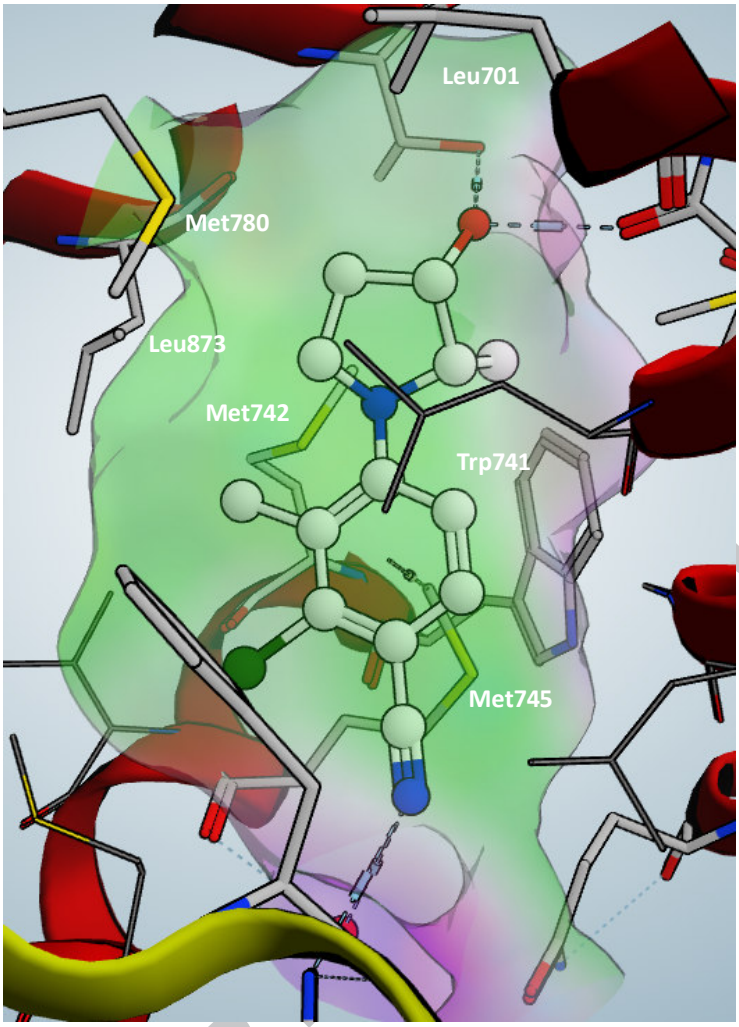
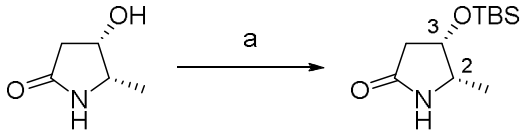


Figure 4. X-ray information of compound **1a** with the LBD of AR.

2. Chemistry

Synthesis of TBS protected pyrrolidine-5-one derivatives **4a** is depicted in Scheme 1. Previously reported (2*S*,3*S*)-3-hydroxy-2-methylpyrrolidine-5-one (**3**)[34] was treated with TBSCl and imidazole to afford **4a**.

Scheme 1. Synthesis of compound **4a**[a]

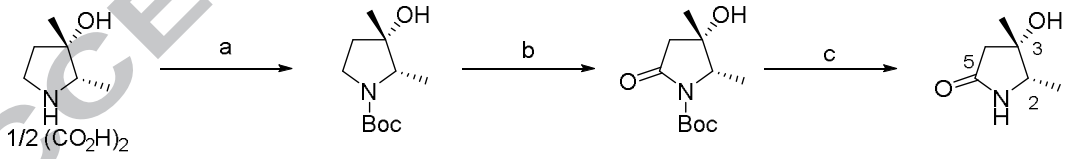


3 **4a**

[a]Reagents and conditions: (a) TBSCl, imidazole, DMF, 74%

2,3-Dimethylpyrrolidin-5-one analog **4b** was synthesized from optically pure (2*S*,3*S*)-2,3-Dimethylpyrrolidin-3-ol 1/2 oxalate (**5**).[34,36] Boc protection of pyrrolidine **5** was carried out to give **6** in 72% yield. The oxidation of pyrrolidine ring by active RuO_4 with cat. RuO_2•H_2O and $NaIO_4$ afford **7** in 34% yield, and the successive de-protection gave **4b** in 94% yield (Scheme 2).

Scheme 2. Synthesis of 2,3-dimethylpyrrolidin-5-one analog **4b**[a]



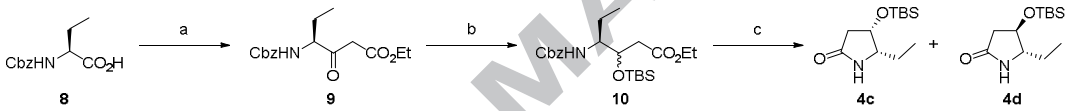
5 **6** **7** **4b**

[a]Reagents and conditions: (a) Boc_2O, 1 M NaOH aq., THF, 72%; (b) cat. RuO_2/H_2O, $NaIO_4$, EtOAc/H_2O, 34%; (c) 4 M HCl in EtOAc, 94%.

The preparation of **4c**, **4d** is shown in Scheme 3. *N*-Cbz-protected (*S*)-2-butanoic acid (**8**)

was activated with CDI, and the subsequent condensation with lithium enolate gave **9** in 62% yield. Diastereo mixtures **10** were prepared by the ketone selective reduction of compound **9** followed by the protection of hydroxyl group using TBSOTf and 2,6-lutidine in 66% yield. *N*-Cbz group was cleaved by hydrogenation with palladium on carbon under hydrogen atmosphere. Successive cyclization by NaOMe and the following chromatographic purification by silica gel provided (2*S*,3*S*)-5-oxopyrrolidine derivative **4c** in 13% and (2*S*,3*R*)-5-oxopyrrolidine derivative **4d** in 59% yield, respectively (Scheme 3). The relative configurations of compounds **4c**, **d** were determined by X-ray and NOE analysis using **2d**[37] and **2e**,[38] respectively (Figure 5).

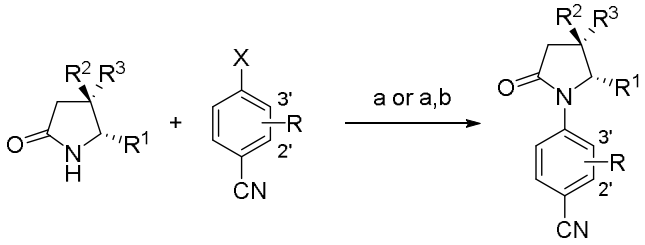
Scheme 3. Synthesis of ethyl analog **4c**,**d**[a]



[a]Reagents and conditions: (a) (1) CDI, THF, (2) *n*-BuLi, *i*-Pr_2NH, EtOAc, THF, 62% for 2 steps; (b) (1) $NaBH_4$, MeOH, (2) TBSOTf, 2,6-lutidine, THF, 66% for 2 steps; (c) (1) Pd/C, H_2, MeOH, (2) NaOMe, MeOH, (3) Chromatographic purification by silica gel, 13% (**4c**) and 59% (**4d**) for 3 steps.

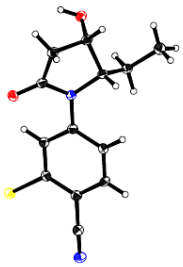
A coupling reaction of pyrrolidine-5-ones **4a–d** with 4'-halobenzonitriles **11** was accomplished by Buchwald reaction using $Pd_2(dba)_3$, Xantphos, cesium carbonate, and the subsequent de-protection of TBS group in the case of **4a, c, d** gave 4'-(5-oxopyrrolidine-1-yl)benzonitrile derivatives **2a–h** in 6–87% yield (Scheme 4).

Scheme 4. Synthesis of compounds **2a**–**h** by Buchwald reaction[a]

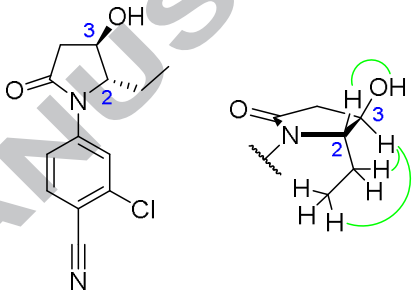


2a: R^1= Me, R^2 = H, R^3 = OH, R = 2'-Cl
2b: R^1= Me, R^2 = H, R^3 = OH, R = 2'-Cl-3'-Me
2c: R^1= Me,R^2 = Me, R^3 = OH, R = 2'-Cl
2d: R^1= Et, R^2 = H, R^3 = OH, R = 2'-Cl
2e: R^1= Et, R^2 = OH, R^3 = H, R = 2'-Cl
2f: R^1 = Et, R^2 = H, R^3 = OH, R = 2'-CF_3
2g: R^1 = Et, R^2 = H, R^3 = OH, R = 2'-OMe
2h: R^1 = Et, R^2 = H, R^3 = OH, R = 2'-Cl-3'-Me

[a]Reagents and conditions: (a) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane; (b) HCl aq., EtOH, THF, 33–86%.



X-ray analysis of Compound **2d**[37]

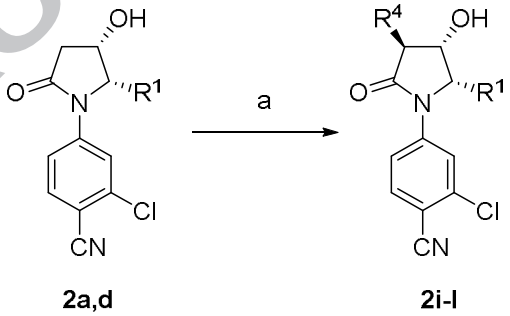


NOE analysis of Comound **2e**[38]

Figure 5. X-ray and NOE analysis of compound **2d** and **2e** to determine the relative structure.

The preparation of 4-alkyl (R^4) pyrrolidine-5-one analog **2i–l** was performed by dilithiation of **2a** and **2d** followed by the selective alkylation in 5–42% yield. The stereoselectivity was caused by steric hindrance of hydroxyl group. The relative configuration of **2i** was determined by NOE analysis (Figure 6).[38]

Scheme 5. Synthesis of 4-alkyl (R^4) pyrrolidine-5-one analog **2g–i**[a]



2i: R^1 = Me, R^4 = Me
2j: R^1 = Me, R^4 = Et
2k: R^1 = Me, R^4 = Bn
2l: R^1 = Et, R^4 = Me

[a]Reagents and conditions: (a) *n*-BuLi, *i*-Pr_2NH, THF then R^4Br or R^4I, 5–42%

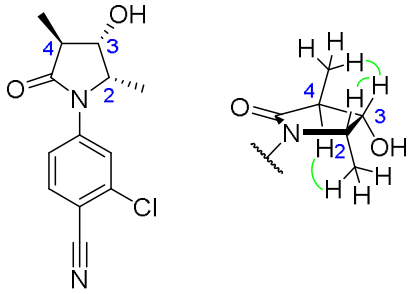
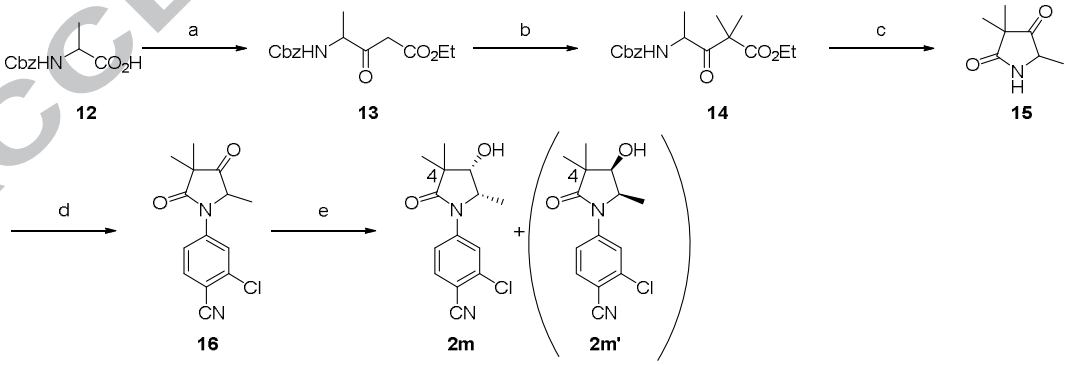


Figure 6. NOE analysis of compound **2i** to determine the relative structure.[38]

4,4-Dimethyl pyrrolidine-5-one analog **2m** was synthesized according to scheme 6. Activation of Cbz-protected amino acids **12** with CDI followed by the condensation with the lithium enolate afforded **13** in 46% yield. Subsequent dimethylation gave **14** in 85% yield, and the following deprotection induced cyclization to produce **15** in 87% yield. Coupling reaction of **16** with 2-chloro-4-bromobenzonitrile under Buchwald reaction conditions gave **18** in 53% yield. *Cis*-selective reduction of **16** with L-Selectride® and the following chiral HPLC purification provided optical pure **2m** in 32% yield (99.9% *ee*) and **2m'** in 34% yield (99.5% ee). The relative configuration of **2m** was determined by NOE analysis,[38] and the absolute configuration was assigned based on their binding and agonistic activities.[39]

Scheme 6. Synthesis of 4-dimethyl analog **2m**[a]



[a]Reagents and conditions: (a) (1) CDI, THF, (2) *n*-BuLi, *i*-Pr_2NH, EtOAc, THF, 46%; (b) MeI,

K_2CO_3, acetone, 85%; (c) Pd/C, H_2, MeOH, 87%; (d) 4-bromo-2-chlorobenzonitrile, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, 53%; (e) (1) 1.0 M L-Selectride® (2) Chiral HPLC purification, 32% for **2m** and 34% for **2m'**, respectively..

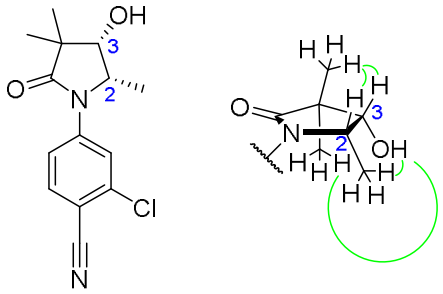
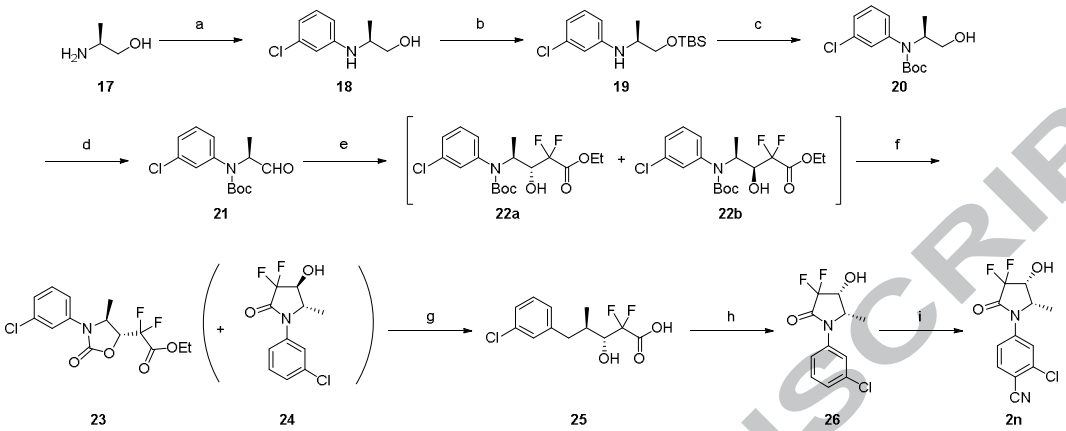


Figure 7. NOE analysis of compound **2m** to determine the relative structure.[38]

4,4-Difluoro-pyrrolidin-5-one analog **2n** was synthesized via Reformatsky reaction as a key reaction (Scheme 7). 3-Chloro-1-iodobenzene was condensed with (*S*)-2-aminopropan-1-ol (**17**) by Ullmann-Goldberg reaction with CuI as a catalyst to afford **18**, which was converted to TBS protected **19** using TBSCl and imidazole. Boc protection of nitrogen atom followed by the selective TBS deprotection with TBAF gave **20** in 64% yield. Following Swern oxidation of primary alcohol **20** provided aldehyde **21** in 96% yield. Reformatsky reaction was carried out for **21** and ethyl 2-bromo-2,2-difluoroacetate with Zn successfully afforded secondary alcohol **22a** and **22b** as diastereo mixtures. Deprotection of Boc group followed by the intramolecular lactamization gave carbamate product **23** and lactam **24** in 38% and 21% yield, respectively. Hydrolysis of desired isomer **23** with aqueous sodium hydroxide was performed to provide carboxylic acid **25**, which was quickly cyclized to give lactam **28** in 97% yield in 2 steps. Iodination of **26** with NIS under acidic condition followed by cyanation using CuCN successfully afforded 4,4-difluoro-5-oxopyrrolidine analog **2n**. Relative configuration of **2n** was determined by X-ray crystal analysis,[40] and high optical purity of **2n** was confirmed by chiral HPLC analysis (99.8% *ee*).[41,42]

Scheme 7. Synthesis of 4,4-difluoro-pyrrolidin-5-one analog **2n**[a]



[a]Reagents and conditions: (a) 1-Chloro-3-iodobenzene, CuI, $(CH_2OH)_2$, K_3PO_4, *i*-PrOH, 80 ℃, 97%; (b) TBSCl, imidazole, DMF, 50 ℃, 97%; (c) (1) *n*-BuLi, THF, −78 ℃; (2) Boc_2O, −78 ℃ to room temperature; (3) TBAF, THF, 64% for 3steps; (d) $(COCl)_2$, DMSO, Et_3N, THF, −60 ℃ to −20 ℃, 96%; (e) $Br(F)_2CCO_2Et$, Zn, THF, reflux; (f) (1) 4 M HCl in EtOAc; (2) *i*-Pr_2NEt, THF, reflux, 38% for 3 steps (21% for **27**); (g) 8 M NaOH aq., MeOH, reflux; (h) MeOH, reflux, 97% for 2 steps; (i) (1) NIS, concentrated H_2SO_4, AcOH; (2) CuCN, NMP, 150 ℃; (3) recrystallization from EtOAc/n-hexane, 41% for 3 steps (99.8% *ee*).

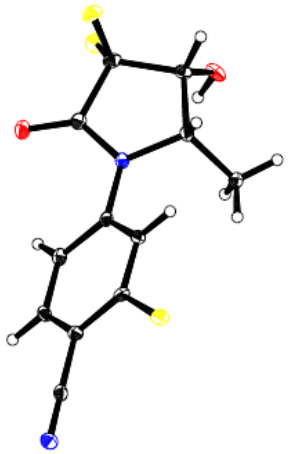
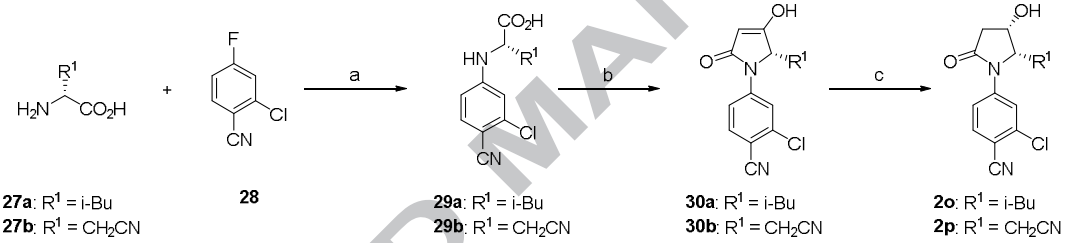


Figure 8. X-ray analysis of compound **2n**.[40]

Further modifications of R^1 at 2-position of pyrrolidine-5-one were performed as shown in Scheme 8. Condensation of amino acids **27a**, **b** with 2-chloro-4-fluorobenzonitrile **28** afforded **29a**, **b** in 72 and 100% yield, respectively. Treatment of **29a**, **b** with Meldrum's acid, and the successive cyclization by refluxing in EtOAc afforded compounds **30a**, **b** in 32 and44%, respectively. 4-(5-Oxopyrrolidine-1-yl)benzonitrile derivatives **2o, p** were synthesized by *cis*-selective reduction using $NaBH_4$ and AcOH in 59 and 62% yield, respectively. The relative configuration of **2o** was confirmed by X-ray analysis, and that of **2p** was determined based on the configuration of **2o** (Figure 9).[43]

Scheme 8. Synthesis of various 3-alkyl (R^1) pyrrolidine-5-one analog **2o**, **p**[a]



[a]Reagents and conditions: (a) Cs_2CO_3, DMF, 72–100%; (b) (1) Meldrum's acid, CDI, DMAP, THF, (2) EtOAc, reflux, 32–44% for 2 steps; (c) $NaBH_4$, AcOH, CH_3CN, 59–62%.

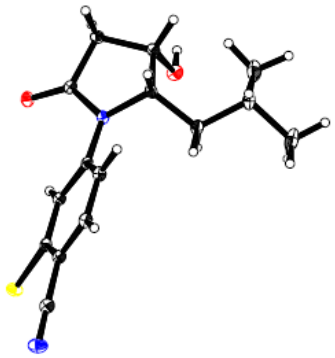
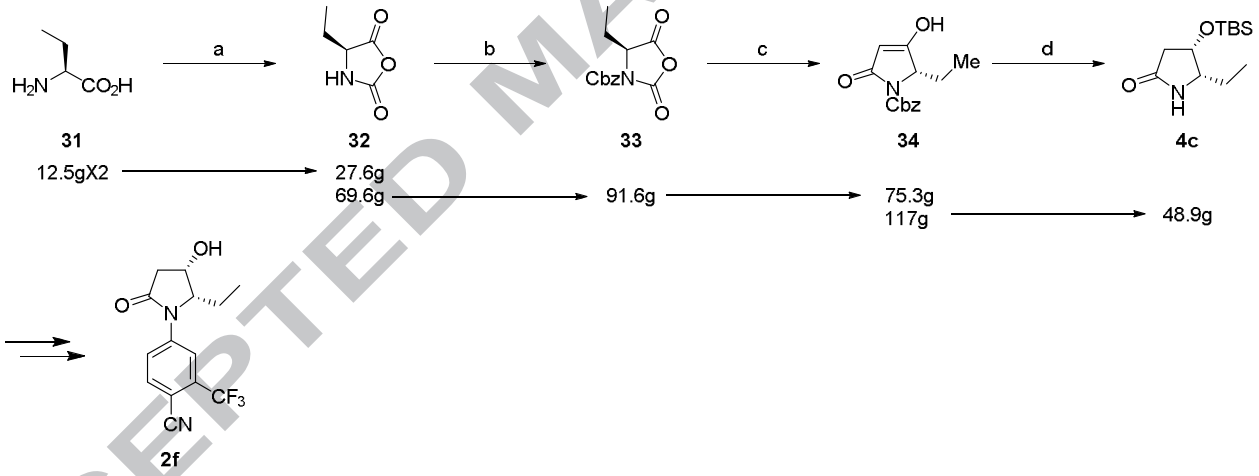


Figure 9. X-ray analysis of compound **2o** to determine the relative structure.[43]

Stereo selective synthesis of (2*S*,3*S*)-5-oxopyrrolidine derivative **2f** was performed for in

vivo study, because the separation of the compound **2c** and **2d** was quite difficult by silica gel column chromatography. Activation of *N*-Cbz-protected (*S*)-2-butanoic acid (**31**) with triphosgene followed by the cyclization gave **32** in 58% yield. Highly reactive compound **32** was condensed with Medrum's acid and subsequent cyclization afforded compound **33** in 83% yield. *Cis*-selective reduction of **33** was performed with sodium tetrahydroborate-AcOH. Following protection of hydroxyl group with TBS and the subsequent cleavage of Cbz provided **4c** in 19% yield (Scheme 9). Finally, bulk synthesis of compound **2f** was completed according to a similar synthetic procedure with Scheme 4. The optical purity of the compound **2f** was confirmed by HPLC analysis.[44,45]

Scheme 9. *Cis*-selective synthesis of ethyl analog **2f**[a]



[a]Reagents and conditions: (a) triphosgene, THF, 50 °C, 89%; (b) benzyl chloroformate, *N*-methylmorpholine, 0 °C, 65%; (c) (1) Meldrum's acid, Et_3N, THF, (2) EtOAc, reflux, 83% for 2 steps; (d) (1) $NaBH_4$, AcOH, quant.; (2) TBSCl, imidazole, DMF, (3) Pd/C, H_2, MeOH, 45% for 3 steps.

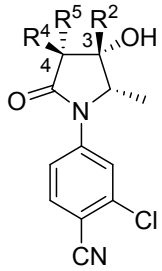
3. Results and discussion

3.1. AR binding and reporter gene assay

AR binding affinities were evaluated by competitive displacement of a radiolabeled [^{3}H] mibolerone from AR, and the data were reported as IC_{50} values. Functional activities were determined by luciferase activities and described as EC_{50} values.

Initially, we evaluated the effects of the substituents at 4-position of the 5-oxopyrrolidine ring (Table 1). Amazingly, just the introduction of methyl group (**2i**) at *trans*-position (R^4) enhanced the binding affinity by 10 times (IC_{50} = 1.3 nM), and agonistic activities by 800 times (EC_{50} = 1.0 nM) compared with **2a**. Ethyl group (**2j**) also enhanced the activity, however, benzyl group (**2k**) did not contribute to increasing the potency. 4,4-Dimethyl (**2m**, EC_{50} = 30 nM) and 4,4-difluoro derivatives (**2n**, EC_{50} = 12 nM) showed medium agonistic activities. These results indicates that there is a small and lipophilic pocket around the 4-position, especially *trans*, of the 5-oxopyrrolidine ring. Contrary to our expectations based on the SAR obtained in the series of compound **1b**, installation of methyl group at the 3-position of the 5-oxopyrrolidine ring (R^2) was not effective to improve agonistic activity (**2c**, EC_{50} = 150 nM).[34]

Table 1. Binding inhibitory and agonistic activities of 4-(5-oxopyrrolidine-1-yl)benzonitrile derivatives.



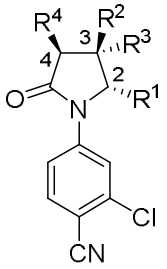
Compd.	R^4	R^5	R^2	AR binding[a]	AR reporter[a]

				IC_{50} (nM)[b]	EC_{50} (nM)[b]
2a	H	H	H	7.8 (5.7-11)	810 (500-1300)
2i	Me	H	H	1.3 (1.2-1.4)	1.0 (0.70-1.5)
2j	Et	H	H	3.2 (2.6-4.1)	3.6 (2.2-5.9)
2k	Bn	H	H	6.0 (5.1-7.0)	220 (160-290)
2m	Me	Me	H	14 (11-17)	30 (19-47)
2n	F	F	H	1.1 (0.90-1.3)	12 (7.0-19)
2c	H	H	Me	15 (12-18)	150 (59-370)

[a]Human AR was used. [b]IC_{50} and EC_{50} values are presented as means of duplicate experiments, with 95% confidence intervals (95%CI) in parentheses.

We moved on to the modification of R^1 at the 2-position of the 5-oxopyrrolidine ring (Table 2). Surprisingly, replacement of a methyl group (**2a**) with an ethyl group (**2d**) led to a 130-fold increase in the agonistic activity (EC_{50} = 6.0 nM). On the other hand, an isobutyl group was not effective to enhance agonistic activity (**2o**, EC_{50} = 170 nM). Introduction of a polar cyanomethyl group (**2p**) resulted in significant loss of agonistic activity. These findings suggest that the space around the 2-position is small and hydrophobic. Based on these results, we performed additional modification of compound **2d**. As expected, compound **2l** with *trans*-4-methyl group showed greatly strong agonistic activity (EC_{50} = 0.39 nM). (*R*)-OH group also increased the agonistic activity (**2e**, EC_{50} = 8.0 nM), which was a similar trend shown in the series of naphthonitrile compounds.[46]

Table 2. Binding inhibitory and agonistic activities of 4-(5-oxopyrrolidine-1-yl)benzonitrile derivatives.

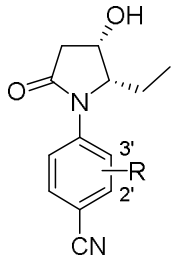


Compd.	R^1	R^4	R^2	R^3	AR binding[a] IC_{50} (nM)[b]	AR reporter[a] EC_{50} (nM)[b]
2a	Me	H	H	OH	7.8 (5.7-11)	810 (500-1300)
2d	Et	H	H	OH	3.9 (2.7-5.6)	6.0 (4.3-8.4)
2o	i-Bu	H	H	OH	5.5 (4.7-6.5)	170 (69-410)
2p	CH_2CN	H	H	OH	14 (10-20)	>10000
2l	Et	Me	H	OH	1.8 (1.6-2.1)	0.39 (0.29-0.52)
2e	Et	H	OH	H	3.4 (2.6-4.5)	8.0 (5.3-12)

[a]Human AR was used. [b]IC_{50} and EC_{50} values are presented as means of duplicate experiments, with 95% confidence intervals (95%CI) in parentheses.

Then, we examined the effects of substituents on the benzonitrile (R). 2'-Trifluomethyl derivative **2f** showed highly potent agonistic activity (EC_{50} = 4.7 nM), comparable with **2d**. 2'-Methoxy (**2g**, EC_{50} = 24 nM) and 2'-chloro-3'-methyl (**2h**, EC_{50} = 23 nM) groups resulted in decrease of agonistic activity.

Table 3. Binding inhibitory and agonistic activities of 4-(5-oxopyrrolidine-1-yl)benzonitrile derivatives.



Compd.	R	AR binding[a] IC_{50} (nM)[b]	AR reporter[a] EC_{50} (nM)[b]
2d	2'-Cl	3.9 (2.7-5.6)	6.0 (4.3-8.4)
2f	2'-CF_3	3.6 (2.5-5.0)	4.7 (3.3-6.8)
2g	2'-OMe	9.2 (6.8-13)	24 (18-31)
2h	2'-Cl-3'-Me	9.0 (6.9-12)	23 (16-34)

[a]Human AR was used. [b]IC_{50} and EC_{50} values are presented as means of duplicate experiments, with 95% confidence intervals (95%CI) in parentheses.

3.2. Tissue selectivity in *in vivo* study

Tissue selectivity was investigated using the Hershberger assay.[47] In this assay, the weight of the levator ani muscle was used as an indicator of anabolic effects, and the weights of the prostate was used as an indicator of androgenic activity. The compounds (2.5 mg/kg, bid, po) were administered to immature castrated male SD rats (4 weeks old) for 4 days. To extrapolate this rat assay to a human study, testosterone propionate (TP, 0.5 mg/kg/day, sc, qd) was administered along with the test compounds to complement adrenal testosterone, which is not produced in rats. In this type of study, TP was reported to have equivalent potency and efficacy for the prostate and levator ani muscle when dosed subcutaneously.

Compound **2g** with weak agonistic activity (EC_{50} = 24 nM) did not show any effects in this assay. On the other hand, another weak agonist **2c** (EC_{50} = 150 nM) reduced the weight of

prostate. Compound **2c** showed extremely good pharmacokinetic profiles, which would be the reason for the potent efficacy in this assay.[48] This result indicates that weak agonists can show antagonistic effects on prostate if the enough amount of compound was delivered. Compounds with strong agonistic activities, **2n** (EC_{50} = 12 nM), **2d** (EC_{50} = 6.0 nM), **2e** (EC_{50} = 8.0 nM) and **2f** (EC_{50} = 4.7 nM), demonstrated tissue-selective in vivo pharmacological activity. These compounds increased the weight of the levator ani muscle up to about 130% relative to that in TP-treated vehicle rats. On the other hand, these 4 compounds did not influence the weight of the prostate. In contrast, the highly strong agonist **2l** (EC_{50} = 0.39 nM) increased levator ani muscle and prostate, indicating that this compound works as just an agonist to the both tissues.. Through this *in vivo* study we confirmed that compound **2n,d,e,f** were SARM having tissue selective pharmacological activity to levator ani and prostate.

The mechanism for the tissue selectivity was not clearly elucidated, however, there are some publications that shows the ability to recruit cofactors will be different between SARMs and pure agonist, such as testosterone.[49,50]

Table 4. Tissue selectivity in vivo (3-week old immature rats, 2,5 mg/kg, bid, po, 4-days treatment).

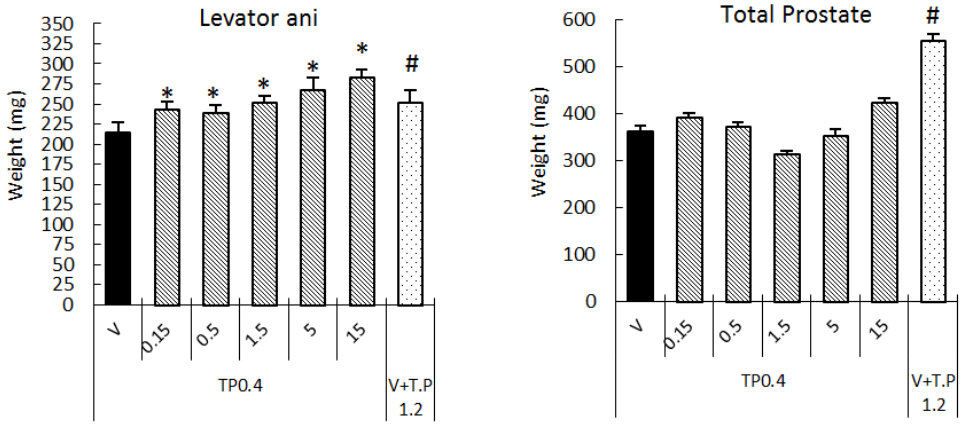
Compd.	AR binding[a]	AR reporter[a]	Tissue Selectivity (%)[c]	
	IC_{50} (nM)[b]	EC_{50} (nM)[b]	Levator ani	Prostate
2g	9.2 (6.8-13)	24 (18-31)	114	90
2c	15 (12-18)	150 (59-370)	116	76*
2n	1.1 (0.90-1.3)	12 (7.0-19)	139*	85
2d	3.9 (2.7-5.6)	6.0 (4.3-8.4)	134*	94
2e	3.4 (2.6-4.5)	8.0 (5.3-12)	124*	84
2f	3.6 (2.5-5.0)	4.7 (3.3-6.8)	129*	106

2l	1.8 (1.6-2.1)	0.39 (0.29-0.52)	161*	149*

[a]Human AR was used. [b]IC_{50} and EC_{50} values are presented as means of duplicate experiments, with 95% confidence intervals (95%CI) in parentheses. [c]The values shown are the values compared with the control group (TP, 0.5 mg/kg/day, sc, qd). *$p \leq 0.05$ (Student's t-test) when compared with the TP (0.5 mg/kg)-treated control group (n=5).

We selected **2f** for the confirmation of dose dependency in vivo. Compound **2f** (0.15, 0.50, 1.5, 5.0, 15 mg/kg/day, qd, po) was administered to 8-weeks-old castrated male SD rats for 7 days along with TP (0.4 mg/kg/day, qd, sc). Compound **2f** exerted significant effects on levator ani muscle at the minimal dose (0.15 mg/kg/day), and the efficacy increased in a dose dependent manner, without influencing the weight of prostate even at the highest dose (15 mg/kg/day). Based on this study, we chose compound **2f** for further development.

Figure 10. Dose dependency for tissue selectivity in vivo (8-weeks-old SD rats, 7 days treatment).



*$p \leq 0.05$ (Dunnett test) when compared with the TP (0.4 mg/kg)-treated control group (n = 5).

$p \leq 0.05$ (T-test) when compared with the TP (0.4 mg/kg)-treated control group

3.3. Sexual behavior induction assay

To confirm the agonistic activity on the CNS, a sexual behavior induction assay was performed (Table 5). Selected fertile male rats were castrated to eliminate sexual behavior. After treatment with **2f** for 3 weeks, sexual behavior induction was confirmed by the pseudopregnancy rate in female rats. Compounds **2f** induced sexual behavior at the minimal dose (0.5 mg/kg/day, qd, po). These results proved that **2f** could act as androgen agonist on the CNS.

Table 5. Sexual behavior induction assay

Compound	Dose (mg/kg, qd, for 21 days)				
	0	0.5[a]	1.5[a]	5.0[a]	3.0[b]
2f	0	100%*	100%*	86%*	/
TP (3 mg/kg, sc)	/	/	/	/	100%*

[a]po [b]sc [*]induction rate of pseudopregnancy (% of total number), $p \leq 0.05$ (Fisher's exact test-test) when compared with the vehicle-treated control group ($n = 7$).

3.4. PK profiles

Pharmacokinetic profiles in rats, dogs and monkeys were evaluated, and the results are shown in Table 6. Compound **2f** displayed good oral bioavailability (B.A.) in all species [rats (74.6%), dogs (76.6%) and monkeys (48.1%)]. In addition, **2f** was found to show good metabolic stabilities with little species difference between rat, dog, mouse, monkey and human (Table 7). These data suggest that PK profiles of **2f** correlate with its metabolic stabilities in vitro, which indicates that compound **2f** is expected to show good PK profiles also in human.

Table 6. Pharmacokinetic parameters of **2f**[a]

Animal	iv (0.1 mg/kg)

	C_{5min} (ng/mL)	AUC_{0-24h} (ng•h/mL)	MRT (h)	$Vd_{(ss)}$ (mL/kg)	CL_{total} (mL/h/kg)
rat[b]	70.8	108.5	2.32	2167	934
dog[c]	106.2	150.5	4.84	3297	681
monkey[d]	72.9	118.3	1.72	1469	853

	po (1 mg/kg)				
Animal	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24h} (ng·h/mL)	MRT (h)	B.A. (%)
rat[b]	218.1	0.50	809.4	3.06	74.6
dog[c]	264.9	0.75	1159.5	4.34	76.6
monkey[d]	71.2	1.67	557.8	5.21	48.1

[a]Data are expressed as the mean ± S.D. (n = 3). [b]Crl:CD(SD) rat (male). [c]Beagle dog (male). [d]Cynomolgus monkey (male).

Table 7. Metabolic stabilities of compound **2f**.

Animal	Metabolic stability (μL/min/mg)
rat	21
dog	4
mouse	3
monkey	5
human	1

3.5. Nuclear receptor selectivity

Nuclear receptor selectivity against MR (Mineralocorticoid Receptor), GR (Glucocorticoid Receptor), ER (Estrogen Receptor), PR (Progesterone Receptor) was examined, and the results are shown in Table 8. These data indicate that **2f** is highly specific for androgen receptor.

Table 8. Nuclear receptor selectivity of **2f**

	Binding[a] (IC_{50}[b], nM)				Reporter[a] (EC_{50}[b], nM)	
Compound	AR	MR	GR	ER	AR	PR
2f	6.3	>10000	>10000	>10000	7.6	>10000
Dihidrotestosterone	0.67	360	540	7300	3.0	8200

[a]Human AR was used (n = 2). [b]IC_{50} and EC_{50} values shown are the mean values of duplicate

measurements (n = 2).

3.6. Toxicological study

Two-week oral gavage toxicity studies were conducted in rats, dogs and monkeys. All the findings are related to the expected pharmacology except for convulsion and myocardial necrosis in monkeys at the plasma exposure more than 200 times higher than that at efficacy dose level. No effects on electrocardiography, heart rate and mean blood pressure were noted in anesthetized beagle dogs after the single intravenous dose at supra-pharmacologic exposures. The true CV risks will be evaluated in the clinical study.[51,52]

3.7. Co-crystal structure of 2f with LBD of androgen receptor.

To determine the binding mode, we solved the X-ray co-crystal structure of compound **2f** with AR (Figure 11). The co-crystal structure of **2f** with AR ligand binding domain, residues 671–919, was obtained at 1.4 Å resolutions. This data supports our hypothesis that there are small and hydrophobic pockets around the 2- and 4-position of the 5-oxopyrrolidine ring. Trp741, Met742, Met745 constructs a small and lipophilic pocket around 2-position, and Leu701, Met780, Leu873 constituted the pocket around 4-positon. Especially, the pocket around 4-position exists in *trans* direction, which supports the high potency of *trans*-4-methyl compounds **2i**,**l**. The binding mode of **2f** is almost the same as that of previously reported compound **1a** and **1b**.[34] Binding of the compound **2f** binding with AR is stabilized by hydrogen bonding on both ends of the molecule. It is stabilized by water mediated HBs (hydrogen bonds) to the side chain of Arg752 and the backbone carbonyl of Met745. The other end of the molecule forms HBs between the hydroxyl group on the 5-oxopyrrolidine ring and the side chain oxygen atom of Asn705. Compound **2f** does not form a direct HB to

Thr877, even though it potentially could with an alternate rotamer.

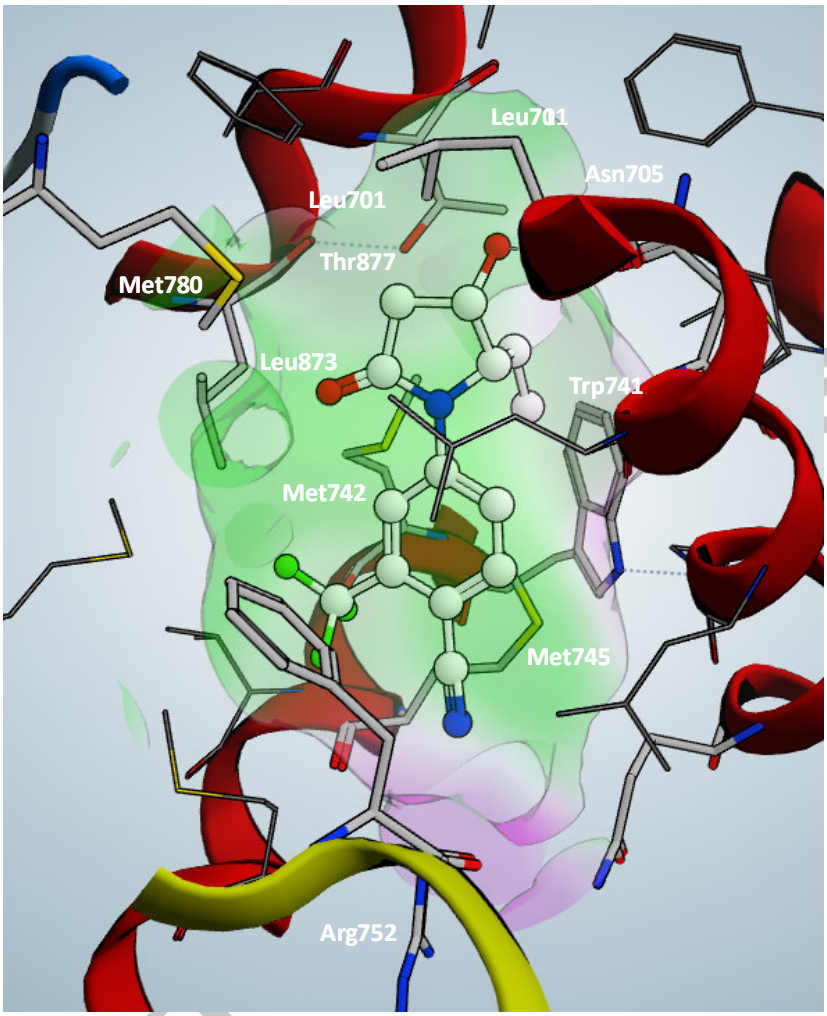


Figure 11. Co-crystal structure of **2f** with AR.

4. Conclusion

With the aim of developing SARMs, we modified the substituents on the 5-oxopyrrolidine ring of compound **2a** considering the co-crystal structure of compound **1a** with AR. We enhanced the agonistic activities more than 100 times by modification of **2a**. As a result of further investigation, we found three types of compounds with different tissue selectivity: (1) Antagonistic effect on prostate (**2b**), (2) Agonistic effect on muscle and no effect on prostate (**2n,d,e,f**), (3) Agonistic effects on both muscle and prostate (**2l**). Of those compounds, the compound **2f** showed good tissue selectivity in a dose dependent manner In addition, compound **2f** induced sexual behavior in castrated rats, which suggests that the compound could also act as an agonist on the CNS. Compound **2f** showed good pharmacokinetic profiles *in vivo* as well as *in vitro* metabolic stabilities with a little species difference, which indicates that compound **2f** is expected to show good PK profiles in human. Compound **2f** also has highly specific nuclear receptor selectivity and acceptable toxicological profiles. Finally, our hypothesis to improve the agonistic activity was confirmed by co-crystal structure of **2f** with LBD of AR. Further exploratory studies using **2f** are under way, and the results will be reported in due course.

5. Experimental section

5.1. Chemistry

Melting points were determined on a Büchi melting point apparatus and were not corrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini-200 (200 MHz), a Varian Mercury-300 (300 MHz), a Bruker DPX300 (300 MHz) or a Bruker Avance II+ 600 (600 MHz) instrument. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated solution. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; dt, doublet of triplet; br s, broad singlet; m, multiplet. Coupling constants (*J* values) are given in hertz (Hz). Elemental analyses were performed by Takeda Analytical Laboratories. Reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). Chromatographic purification was performed on silica gel columns 60 (0.063–0.200 mm or 0.040–0.063 mm, Merck), basic silica gel (ChromatorexNH, 100–200 mesh, Fuji Silysia Chemical Ltd.), or Purif-Pack (SI 60 μM or NH 60 μM, Fuji Silysia, Ltd.). Commercial reagents and solvents were used without additional purification. Compounds **2a–p** were confirmed to be pure by elemental analysis. The yields were not optimized. The following abbreviations are used. Boc_2O = di-*tert*-butyl dicarbonate, CDI = *N*,*N*′-carbonyldiimidazole, DIEA = diisopropylethylamine, DMAP = 4-dimethylaminopyridine, DMF = *N*,N-dimethylformamide, DMSO = dimethyl sulfoxide, Et_3N = triethylamine, Et_2O = diethyl ether, EtOAc = ethyl acetate, EtOH = ethanol, IPE = diisopropyl ether, L-Selectride® = lithium tri-*s*-butylhydroborate, Meldrum's acid = 2,2-dimethyl-1,3-dioxane-4,6-dione, MeOH = methanol, NIS = *N*-iodosuccinimide, NMP = *N*-methylpyrrolidone, $Pd_2(dba)_3$ = tris(dibenzylideneacetone)dipalladium(0), i-PrOH =

isopropanol, TBAF = tetrabutylammonium fluoride, TBSCl = *tert*-butyldimethylsilyl chloride, THF = tetrahydrofuran, Xantphos = 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene.

(2*S*,3*S*)-3-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-methylpyrrolidin-5-one (4a). To a solution of (2*S*,3*S*)-3-hydroxy-2-methylpyrrolidin-5-one (**3**) (14.8 g, 129 mmol) in THF (50 mL), imidazole (10.5 g, 154 mmol) and *tert*-butyldimethylsilyl chloride (21.3 g, 141 mmol) was added at 0 °C and the mixture was stirred at room temperature for 18 h. Then the mixture was poured into water and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 2:1 to 0:1) to give **4a** (21.9 g, 95.5 mmol, 74%) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.07 (6H, s), 0.90 (9H, s), 1.17 (3H, d, *J* = 6.3 Hz), 2.27 (1H, dd, *J* = 16.5, 4.2 Hz), 2.52 (1H, dd, *J* = 16.5, 9.3 Hz), 3.70-3.80 (1H, m), 4.37-4.45 (1H, m), 6.00 (1H, br s).

(2*S*,3*S*)-*tert*-Butyl 3-hydroxy-2,3-dimethylpyrrolidine-1-carboxylate (6). To a solution of (2*S*,3*S*)-2,3-dimethylpyrrolidin-3-ol 1/2 oxalate (**5**) (3.31 g, 20.7 mmol) in THF (50 mL) were added 1 M aqueous NaOH solution (40 mL, 40 mmol) and a solution of Boc_2O (5.42 g, 24.8 mmol) in THF (10 mL) at 0 °C. After stirring at room temperature for 20 h, water (50 mL) was added to the reaction mixture at room temperature. The resulting mixture was extracted with EtOAc twice. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane: EtOAc = 9:1 to 3:2) to give **6** (3.21 g, 14.9 mmol, 72%) as a white powder. Furthermore, the recrystallizaion from EtOAc/n-hexane gave **6** (2.43 g, 11.4 mmol, 55%) as colorless prisms. Mp 109–112 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (3H, d, *J* =

6.4 Hz), 1.33 (3H, s), 1.46 (9H, s), 1.66 (1H, s), 1.73–1.85 (1H, m), 1.86–1.98 (1H, m), 3.40 (2H, dd, *J* = 7.2, 6.7 Hz), 3.46–3.57 (1H, m). IR (KBr) 3430, 2965, 2936, 2878, 1688, 1472, 1402, 1338, 1252, 1216, 1170, 1091, 1019, 988, 934, 919 cm^{-1}. Anal. Calcd for $C_{11}H_{21}NO_3$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.46; H, 9.76; N, 6.46.

(2*S*,3*S*)-*tert*-Butyl 3-hydroxy-2,3-dimethyl-5-oxopyrrolidine-1-carboxylate (7). To a solution of **6** (3.05 g, 14.2 mmol) in EtOAc (45 mL) and H_2O (68 mL) were added $RuO_2 \cdot H_2O$ (566 mg, 4.25 mmol) and $NaIO_4$ (4.57 g, 21.3 mmol) at room temperature. After stirring at room temperature for 1.5 days, the organic layer was separated, and the aqueous layer was extracted with EtOAc. To the combined organic layers was added *i*-PrOH (0.7 mL). The resulting mixture was dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane: EtOAc = 9:1 to 3:2) to give **7** (1.11 g, 4.83 mmol, 34%) as a white powder. Furthermore, the recrystallizaion from EtOAc/n-hexane gave **7** as colorless prisms. Mp 122–125 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (3H, d, *J* = 6.4 Hz), 1.46 (3H, s), 1.54 (9H, s), 1.72 (1H, s), 2.47 (1H, d, *J* = 17.0 Hz), 2.73 (1H, d, *J* = 17.0 H), 3.91 (1H, q, J = 6.4 Hz); IR (KBr) 3465, 2970, 2935, 2874, 1773, 1680, 1539, 1473, 1457, 1370, 1339, 1287, 1257, 1161, 1083, 1053, 1035, 1024, 1005, 966, 935 cm^{-1}. Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.61; H, 8.33; N, 5.93.

(2*S*,3*S*)-3-Hydroxy-2,3-dimethylpyrrolidin-5-one (4b). A mixture of **7** (1.00 g, 4.36 mmol) and 4 M HCl solution in EtOAc (10 mL, 40 mmol) was stirred at room temperature for 14 h. Then, the mixture was concentrated in vacuo. The residue was purified by recrystallization form *i*-PrOH/*n*-hexane to give **4b** (309 mg, 2.40 mmol, 55%) as pale red prisms. The filtrate was concentrated in vacuo. The residue was purified using silica gel column chromatography

(EtOAc:MeOH = 1:0 to 9:1) to give **4b** (220 mg, 1.70 mmol, 39%, total 529 mg, 4.10 mmol, 94%) as a white powder. Mp 169–173 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (3H, d, *J* = 6.4 Hz), 1.20 (3H, s), 2.06 (1H, d, *J* = 16.2 Hz), 2.23 (1H, d, *J* = 16.2 Hz), 3.33 (1H, q, *J* = 6.4 Hz), 4.70 (1H, br s), 7.48 (1H, br s); IR (KBr) 3390, 3191, 2955, 2912, 2858, 1675, 1436, 1377, 1332, 1311, 1258, 1222, 1145, 1125, 1105, 1092, 1019, 939 cm^{-1}. MS (*m/z*): 130 $(M + H)^+$. Anal. Calcd for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84. Found: C, 55.62; H, 8.42; N, 10.72.

Ethyl (4*S*)-4-{[(benzyloxy)carbonyl]amino}-3-oxohexanoate (9). To a solution of diisopropylamine (44.3 mL, 311 mmol) in THF (100 mL), 1.6 M n-butyl lithium solution in hexane (198 mL, 317 mmol) was added dropwise at −78 °C. After stirring at −78 °C for 1 h, EtOAc (30.9 mL, 316 mmol) was added dropwise at −78 °C. The mixture was stirred at -78 °C for 1 h, and then a solution of the compound, which was prepared from (2*S*)-2-{[(benzyloxy)carbonyl]amino}butanoic acid (**8**, 25.0 g, 105 mmol) and carbonyldiimidazole (20.5 g, 126 mmol)] in THF (100 mL), was added dropwise to the mixture at −78 °C. After stirring at −78 °C for 1 h, acetic acid (25 mL) was added to quench the reaction and warmed up to room temperature. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane to *n*-hexane:EtOAc = 2:1) to give **9** (20.0 g, 65.1 mmol, 62%) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, t, *J* = 7.5 Hz), 1.27 (3H, t, *J* = 7.2 Hz), 1.60–1.72 (1H, m), 1.91–2.09 (1H, m), 3.47–3.61 (2H, m), 4.11–4.25 (2H, m), 4.40–4.51 (1H, m), 5.11 (2H, s), 5.41 (1H, d, *J* = 7.0 Hz), 7.31–7.39 (5H, m).

(2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-ethylpyrrolidin-5-one (4c) and

(3*R*,2*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-ethylpyrrolidin-5-one (4d). To a solution of **9** (70.7 g, 230 mmol) in MeOH (500 mL) was added sodium borohydride (9.66 g, 255 mmol) at −78 °C, and the mixture was stirred at −78 °C for 1 h and at room temperature for 1 h. Saturated aqueous NH_4Cl was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (EtOAc) to give ethyl (4*S*)-4-{[(benzyloxy)carbonyl]amino-3-hydroxyhexanoate (63.2 g, 204 mmol, 89%). MS (*m/z*): 310 (M + H). To a solution of ethyl (4*S*)-4-{[(benzyloxy)carbonyl]amino-3-hydroxyhexanoate (63.2 g, 204 mmol) and 2,6-lutidine (47.6 mL, 423 mmol) in THF (800 mL) was added *tert*-butyldimethylsilane trifluoromethanesulfonate (70 mL, 305 mmol) at 0 °C. After warming to room temperature, the mixture was stirred for 1 h. Water was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 1:0 to 1:1) to give ethyl (4*S*)-4-{[benzyloxy]carbonyl}amino}-3-(*tert*-butyldimethylsilyloxy)hexanoate (**10**) as a colorless oil (64.0 g, 151 mmol, 74%). MS (*m/z*): 424 $(M + H)^+$. To a solution of **10** (64.0 g, 151 mmol) in MeOH (500 mL) was added 10% Pd/C (50% wet, 6.5 g), and the mixture was stirred at room temperature for 3.5 h under hydrogen atmosphere. The insoluble portions were filtered off through a pad of Celite. Sodium methoxide (13.6 g, 252 mmol) was added to the filtrate, and the mixture was stirred at room temperature for 1 h. Water was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 20:1 to 1:4) to give **4c** (4.64 g, 19.1 mmol, 13%) as a colorless oil, and **4d** (21.6 g, 88.8 mmol, 59%) as a colorless solid.

(2*S*,3*S*)-3-(*tert*-butyldimethylsilyloxy)-2-ethylpyrrolidin-5-one (4c): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.068 (3H, s), 0.070 (3H ,s), 0.89 (9H, s), 0.95 (3H, t, *J* = 7.5Hz), 1.43-1.76 (2H, m), 2.26 (1H, dd, *J* = 16.7, 4.2 Hz), 2.51 (1H, dd, *J* = 16.7, 6.3 Hz), 3.43-3.56 (1H, m), 4.39–4.50 (1H, m), 5.94 (1H, brs).

(3*R*,2*S*)-3-(*tert*-butyldimethylsilyloxy)-2-ethylpyrrolidin-5-one (4d): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.07 (3H, s), 0.08 (3H, s), 0.96 (3H, t, *J* = 7.5 Hz), 1.33–1.54 (1H, m), 1.54–1.71 (1H, m), 1.54–1.71 (1H, m), 2.26 (1H, dd, *J* = 16.9, 4.6 Hz), 2.60 (1H ,dd, *J* = 16.9, 6.9 Hz), 3.30–3.41 (1H, m), 4.05–4.18 (1H, m), 5.71 (1H, brs).

2'-Chloro-4'-[(2*S*,3*S*)-3-hydroxy-2-methyl-5-oxopyrrolidin-1-yl]benzonitrile (2a) The mixture of **4a** (1.50 g, 6.54 mmol), 4-bromo-2-chlorobenzonitrile (1.85 g, 8.55 mmol), cesium carbonate (3.20 g, 9.82 mmol), $Pd_2(dba)_3$ (299 mg, 0.327 mmol) and Xantphos (378 mg, 0.653 mmol) in dioxane (30 mL) was stirred at 80 °C for 12 h under argon atmosphere. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 20:1 to 4:1) to give crude product. Then the crude product was dissolved with ethanol (20 mL) and THF (20 mL), followed by the addition of concentrated aqueous HCl (5.45 mL, 65.4 mmol). After stirring at room temperature for 2 h, the mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 20:1 to 0:1) and recrystallyzed from EtOAc/hexane to give **2a** (0.980 g, 3.91 mmol, 60%) as a colorless crystal. Mp 161–162 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.32 (3H, d, *J* = 6.3 Hz), 2.00 (1H, d, *J* = 4.5 Hz), 2.68 (1H, dd, *J* = 17.4, 5.4 Hz), 2.85 (1H, dd, *J* = 17.4, 6.6 Hz), 4.30–4.42 (1H,

m), 4.56–4.65 (1H, m), 7.48 (1H, dd, *J* = 8.7, 1.8 Hz), 7.66 (1H, d, *J* = 8.7 Hz), 7.73 (1H, d, *J* = 1.8 Hz). Anal. Calcd for $C_{12}H_{11}ClN_2O_2 \cdot 0.1H_2O$: C, 57.08; H, 4.47; N, 11.10. Found: C, 57.12; H, 4.40; N, 11.07.

2'-Chloro-4'-[(2*S*,3*S*)-3-hydroxy-2-methyl-5-oxopyrrolidin-1-yl]-3'-methylbenzonitrile (2b). This compound was prepared in 33% yield by method similar to that described for **2a**. colorless solid. Mp. 172–173 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.12 (3H, d, *J* = 6.6 Hz), 1.90 (1H, d, *J* = 4.2 Hz), 2.32 (3H, s), 2.59 (1H, dd, *J* = 17.4, 2.1 Hz), 2.87 (1H, dd, *J* = 17.4, 5.7 Hz), 4.10–4.30 (1H, br), 4.50–4.60 (1H, m), 7.00–7.20 (1H, m), 7.56 (1H, d, *J* = 8.1 Hz). Anal. Calcd for $C_{13}H_{13}ClN_2O_2 \cdot 0.2H_2O$: C, 58.19; H, 5.03; N, 10.44. Found: C, 58.24; H, 4.90; N, 10.44.

2'-Chloro-4'-[(2*S*,3*S*)-3-hydroxy-2,3-dimethyl-5-oxopyrrolidin-1-yl]benzonitrile (2c). A test tube was charged with 4-bromo-2-chlorobenzonitrile (615 mg, 2.73 mmol), **4b** (423 mg, 3.27 mmol), Xantphos (241 mg, 409 μmol), $Pd_2(dba)_3$ (125 mg, 136 μmol), Cs_2CO_3 (1.31 g, 3.82 mmol), and 1,4-dioxane (3 mL) at room temperature. The resulting test tube was evacuated and backfilled with argon three times. After stirring at 120 °C for 5 h under microwave irradiated conditions, the reaction mixture was passed through short silica gel column chromatography (EtOAc). The filtrated was concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 9:1 to 2:3), followed by NH silica gel column chromatography (*n*-hexane:EtOAc = 7:3 to 1:4) to **2c** (624 mg, 2.34 mmol, 86%) as a pale yellow powder. Furthermore, the recrystallizaion from EtOAc/*n*-hexane gave **2b** (431 mg, 1.64 mmol, 60%) as a white powder. Mp 93–99 °C. $[\alpha]_D$ = −27.3° (*c* 0.500, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, d, *J* = 6.4 Hz), 1.52 (3H, s), 2.01 (1H, s),

2.63 (1H, d, *J* = 17.0 Hz), 2.79 (1H, d, *J* = 17.0 Hz), 4.07 (1H, q, *J* = 6.4 Hz), 7.46 (1H, d, *J* = 8.7 Hz), 7.67 (1H, d, *J* = 8.7 Hz), 7.70 (1H, s); IR (KBr) 3410, 2977, 2934, 2880, 2230, 1695, 1596, 1549, 1489, 1409, 1362, 1316, 1268, 1237, 1205, 1179, 1123, 1082, 1050, 1037, 973, 947, 913 cm^{-1}. MS (*m/z*): 265 $(M + H)^+$. Anal. Calcd for $C_{13}H_{13}N_2O_2Cl$: C, 58.99; H, 4.95; N, 10.58. Found: C, 59.07; H, 4.89; N, 10.56.

2'-Chloro-4'-[(2*S*,3*S*)-2-ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]benzonitrile (2d). A method similar to that described for **2a** was used to prepare this compound in 18% yield as a colorless solid. Mp 138–140 °C. $[\alpha]_D = -38.4°$ (*c* 0.505, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.02 (3H, t, *J* = 7.3 Hz), 1.69–1.82 (3H, m), 2.67 (1H, dd, *J* = 16.8, 3.9 Hz), 2.85 (1H, dd, *J* = 16.8, 6.9 Hz), 4.06–4.18 (1H, m), 4.62–4.73 (1H, m), 7.40 (1H, d, *J* = 8.5 Hz), 7.59-7.72 (2H, m). Anal. Calcd for $C_{13}H_{13}ClN_2O_2$: C, 58.99; H, 4.95; N, 10.50. Found: C, 58.97; H, 4.80; N, 10.44.

2'-Chloro-4'-[(2*S*,3*R*)-2-ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]benzonitrile (2e). A method similar to that described for **2a** was used to prepare this compound in 87% yield as a colorless solid**.** Mp 118–120 °C. $[\alpha]_D = -37.5°$ (*c* 1.015, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.00 (3H, t, *J* = 7.5Hz), 1.41–1.59 (1H, m), 1.69–1.85 (1H, m), 1.97 (1H, br s), 2.55 (1H, dd, *J* = 18.0, 1.2Hz), 3.01 (1H, dd, *J* = 18.0, 5.9Hz), 4.08 (1H, dd, *J* = 9.3, 3.0Hz), 4.32–4.40 (1H, m), 7.59 (1H, dd, *J* = 8.7, 2.1Hz), 7.66 (1H, d, *J* = 8.7Hz), 7.91 (1H, d, *J* = 2.1Hz). Anal. Calcd for $C_{13}H_{13}ClN_2O_2$: C, 58.99; H, 4.95; N, 10.58. Found: C, 59.01; H, 4.90; N, 10.75.

4'-[(2*S*,3*S*)-2-Ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]-2'-(trifluoromethyl)benzonitrile (2f). A method similar to that described for **2a** was used to prepare this compound (115 mg, 0.386

mmol) by 300 mg (1.23 mmol) of **4a** in 31% yield as a colorless solid. Mp 110–112 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, *J* = 7.5 Hz), 1.71–1.84 (3H, m), 2.64–2.75 (1H, m), 2.81–2.92 (1H, m), 4.14–4.24 (1H, m), 4.66–4.74 (1H, m), 7.70–7.75 (1H, m), 7.83–7.87 (2H, m). Anal. Calcd for $C_{14}H_{13}F_3N_2O_2$: C, 56.38; H, 4.39; N, 9.39. Found: C, 56.42; H, 4.40; N, 9.44.

4'-[(2*S*,3*S*)-2-Ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]-2'-methoxybenzonitrile (2g). A method similar to that described for **2a** was used to prepare this compound in 36% yield as a colorless solid. Mp 158–160 °C. $[\alpha]_D$ = −30.0° (*c* 0.505, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.01 (3H, t, *J* = 7.5 Hz), 1.68–1.82 (3H, m), 2.62–2.70 (1H, m), 2.81–2.90 (1H, m), 3.94 (3H, s), 4.08–4.17 (1H, m), 4.62–4.72 (1H, m), 6.80 (1H, dd, *J* = 8.3, 1.9 Hz), 7.31 (1H, d, *J* = 1.7 Hz), 7.55 (1H, d, *J* = 8.3 Hz). Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.63; H, 6.28; N, 10.79.

2'-Chloro-4'-[(2*S*,3*S*)-2-ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]-3'-methylbenzonitrile (2h). A method similar to that described for **2a** was used to prepare this compound in 6% yield as a colorless solid. Mp 142–146 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.94 (3H, t, *J* = 7.4 Hz), 1.29–1.50 (1H, m), 1.55–1.77 (1H, m), 1.72 (1H, br s), 2.32 (3H, br s), 2.58 (1H, d, *J* = 17.4 Hz), 2.85 (1H, dd, *J* = 17.4, 5.3 Hz), 3.84–4.05 (1H, m), 4.57–4.68 (1H, m), 6.99–7.17 (1H, m), 7.56 (1H, d, *J* = 8.3 Hz). Anal. Calcd for $C_{14}H_{15}ClN_2O_2 \cdot 0.2H_2O$: C, 59.56; H, 5.50; N, 9.92. Found: C, 59.31; H, 5.41; N, 9.69.

2'-Chloro-4'-[(2*S*,3*S*,4*S*)-3-hydroxy-2,4-dimethyl-5-oxopyrrolidin-1-yl]benzonitrile (2i). 1.6 M n-Butyllithium solution in hexane (0.648 mL, 1.04 mmol) was added dropwise to a

solution of diisopropylamine (0.150 mL, 1.07 mmol) in THF (7 mL) at −78 °C. After the completion of the dropwise addition, the mixture was stirred at −78 °C for 1 h. Subsequently, a solution of **2a** (100 mg, 0.399 mmol) in THF (2.5 mL) was added dropwise, and the mixture was further stirred at −78 °C for 30 min. Methyl iodide (0.124 mL, 1.99 mmol) was added dropwise at −78 °C, and the mixture was warmed up to −10 °C for 30 min. Acetic acid (1.0 mL) was added to the mixture to quench the reaction, and the resulting mixture was warmed to room temperature. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 10:1 to 1:3) to give **2i** as a colorless solid (44.0 mg, 0.166 mmol, 42%). Mp. 148–150 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.30–1.36 (6H, m), 1.92 (1H, br s), 2.63–2.76 (1H, m), 4.11–4.25 (1H, m), 4.29–4.43 (1H, m), 7.61–7.67 (2H, m), 7.95 (1H, t, *J* = 1.2 Hz). Anal. Calcd for $C_{13}H_{13}ClN_2O_2$: C, 58.99; H, 4.95; N, 10.58. Found: C, 59.25; H, 4.95; N, 10.41.

2'-Chloro-4'-[(2*S*,3*S*,4*S*)-4-ethyl-3-hydroxy-2-methyl-5-oxopyrrolidin-1-yl]benzonitrile (2j). A solution of diisopropylamine (0.15 mL, 1.07 mmol) in THF (8 mL) was cooled to −78 °C, and 1.6 M n-butyllithium solution in hexane (0.650 mL, 1.04 mmol) was added dropwise. After the completion of the dropwise addition, the mixture was stirred at −78 °C for 1 h. Subsequently, a solution of **2a** (100 mg, 0.399 mmol) in THF (2.0 mL) was added dropwise, and the mixture was further stirred at −78 °C for 1 h. Iodoethane (0.15 mL, 1.88 mmol) was added dropwise at −78 °C, and the mixture was further stirred at −78 °C to −30 °C for 1 h. Acetic acid (1.0 mL) was added to quench the reaction, and the resulting mixture was warmed to room temperature. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was

purified using silica gel column chromatography (hexane:EtOAc = 20:1 to 1:4) to give **2j** as a colorless solid (6.70 mg, 0.0215 mmol, 5.4%). Mp. 109–110 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.10 (3H, t, *J* = 7.5 Hz), 1.32 (3H, d, *J* = 6.0 Hz), 1.61–1.80 (1H, m), 1.81–1.98 (2H, m), 2.53–2.66 (1H, m), 4.26–4.42 (2H, m), 7.56–7.70 (2H, m), 7.93 (1H, d, *J* = 1.5 Hz). MS (*m/z*): 279 $(M + H)^+$.

4'-[(2*S*,3*S*,4*S*)-4-Benzyl-3-hydroxy-2-methyl-5-oxopyrrolidin-1-yl]-2'-chlorobenzonitrile (2k). A method similar to that described for **2k** was used to prepare this compound in 30% yield as a colorless solid. Mp. 99–101 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.25 (3H, d, *J* = 6.6 Hz), 1.48 (1H, d, *J* = 4.2 Hz), 2.58–3.04 (2H, m), 3.25–3.36 (1H, m), 4.11–4.24 (1H, m), 4.25–4.36 (1H, m), 7.21–7.40 (5H, m), 7.51–7.61 (1H, m), 7.61–7.68 (1H, m), 7.88 (1H, d, *J* = 2.1 Hz). Anal. Calcd for $C_{19}H_{17}ClN_2O_2$: C, 66.96; H, 5.03; N, 8.22. Found: C, 66.91; H, 5.09; N, 8.34.

2'-Chloro-4'-[(2*S*,3*S*,4*S*)-2-ethyl-3-hydroxy-4-methyl-5-oxopyrrolidin-1-yl]benzonitrile (2l). A solution of diisopropylamine (0.694 mL, 4.95 mmol) in THF (18 mL) was cooled to −78 °C, and 1.6 M n-butyllithium solution in hexane (2.95 mL, 4.72 mmol) was added dropwise. After the completion of the dropwise addition, the mixture was stirred at −78 °C for 1 h. Subsequently, a solution of **2d** (500 mg, 1.89 mmol) in THF (4 mL) was added dropwise, and the mixture was further stirred at −78 °C for 1 h. Iodomethane (0.59 mL, 9.48 mmol) was added dropwise at −78 °C, and the mixture was stirred between −78 °C and 0 °C for 1.5 h. Water was added, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 10:1 to 1:3) to give **2l** as a colorless solid

(152 mg, 0.545 mmol, 29%). Mp. 124–129 °C. $[\alpha]_D$ = −25.6° (*c* 0.160, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.00 (3H, t, *J* = 7.5 Hz), 1.30 (3H, d, *J* = 7.2 Hz), 1.60–1.80 (1H, m), 1.80–1.99 (1H, m), 2.34 (1H, br s), 2.65–2.81 (1H, m), 4.08–4.31 (2H, m), 7.54 (1H, d, *J* = 8.5 Hz), 7.65 (1H, d, *J* = 8.5 Hz), 7.86 (1H, s). Anal. Calcd for $C_{14}H_{15}ClN_2O_2$: C, 60.33; H, 5.42; N, 10.05. Found: C, 60.19; H, 5.40; N, 9.94.

Ethyl 4-{[(benzyloxy)carbonyl]amino}-3-oxopentanoate (13). To a solution of diisopropylamine (18.8 mL, 134 mmol) in THF (330 mL), 1.6 M n-butyl lithium solution in *n*-hexane (84.0 mL, 134 mmol) was added dropwise at −78 °C. After stirring at −78 °C for 1 h, EtOAc (13.1 mL, 316 mmol) was added dropwise at −78 °C. The mixture was stirred at −78 °C for 1 h, and then a solution of *N*-[(benzyloxy)carbonyl]alanine (**12**) (10.0 g, 44.8 mmol) and carbonyldiimidazole (8.72 g, 53.8 mmol) in THF (100 mL) was added dropwise to the mixture at −78 °C. The mixture was stirred at −78 °C for 1 h. Acetic acid (25 mL) was added to quench the reaction and warmed up to room temperature. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 1:0 to 2:1) to give **13b** (6.11 g, 20.8 mmol, 46%) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.25–1.29 (3H, m), 1.37–1.43 (3H, m), 3.50–3.62 (2H, m), 4.16–4.23 (2H, m), 4.40–4.60 (1H, m), 5.11 (2H, s), 5.30–5.50 (1H, m), 7.32–7.38 (5H, m).

Ethyl 4-{[(benzyloxy)carbonyl]amino}-2,2-dimethyl-3-oxopentanoate (14). The mixture of **13** (6.00 g, 20.5 mmol), iodomethane (3.82 mL, 61.4 mmol) and potassium carbonate (5.65 g, 40.9 mmol) in acetone (150 mL) was refluxed overnight. After cooling to room temperature,

the mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 50:1 to 1:1) to give **14** (5.56 g, 17.3 mmol, 85%) as a colorless oil ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.22 (3H, t, *J* = 7.2 Hz), 1.31 (3H, d, *J* = 6.8 Hz), 1.41 (3H, s), 1.43 (3H, s), 4.06–4.22 (2H, m), 4.74 (1H, dd, *J* = 8.5, 7.2 Hz), 5.02–5.17 (2H, m), 5.23–5.37 (1H, m), 7.28–7.46 (5H, m).

3,3,5-Trimethylpyrrolidine-2,4-dione (15). To a solution of **14** (5.56 g, 17.3 mmol) in methanol (100 mL) was added 10% Pd/C (50% wet, 2.00 g) and the mixture was stirred at room temperature for 18 h under hydrogen atmosphere. The insoluble portions were filtered off through a pad of Celite® and the filtrate was concentrated in vacuo. The resulting solid was washed with hexane to give **15** (2.13 g, 15.1 mmol, 87%) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.26 (3H, s), 1.27 (3H, s), 1.40 (3H, d, *J* = 6.8 Hz), 4.08 (1H, q, *J* = 6.9 Hz), 6.07 (1H, br s).

2-Chloro-4-(3,3,5-trimethyl-2,4-dioxopyrrolidin-1-yl)benzonitrile (16). The mixture of **15** (450 mg, 2.48 mmol), 4-bromo-2-chlorobenzonitrile (828 mg, 3.83 mmol), cesium carbonate (1.56 g, 4.78 mmol), $Pd_2(dba)_3$ (292 mg, 0.319 mmol) and Xantphos (553 mg, 0.956 mmol) in dioxane (15 mL) was stirred at 80 °C for 12 h under argon atmosphere. The mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 20:1 to 4:1) to give **16** (517 mg, 1.70 mmol, 53%) as a colorless solid. Mp. 158–160 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.35 (3H, s), 1.40 (3H, s), 1.46 (3H, d, *J* = 6.8 Hz), 4.61 (1H, q, *J* = 6.9 Hz), 7.55 (1H, dd, *J* = 8.6, 2.1 Hz), 7.72 (1H, d, *J*

= 8.5 Hz), 7.87 (1H, d, *J* = 2.1 Hz). Anal. Calcd for $C_{14}H_{13}ClN_2O_2$: C, 60.77; H, 4.74; N, 10.12. Found: C, 60.96; H, 4.60; N, 10.09.

2'-Chloro-4'-[(2*S*,3*S*)-3-hydroxy-2,4,4-trimethyl-5-oxopyrrolidin-1-yl]benzonitrile (2m). To a solution of **16** (400 mg, 1.45 mmol) in THF (10 mL), 1.0M L-selectride THF solution (2.17 mL, 2.17 mmol) was added at −78 °C under nitrogen atmosphere. After stirring at −78 °C for 0.5 h, the mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 20:1 to 1:1) and recrystallyzed from EtOAc/*n*-hexane to give racemic mixtures (**2m** and **2m'**, 283 mg, 1.02 mmol, 70%) as a colorless solid. The racemic mixtures (44.0 mg, 0.158 mmol) was resolved using HPLC to afford optically pure **2m** (20.0 mg, 71.8 μmol, 45%, 99.9%ee) and **2m'** (22.0 mg, 78.9 μmol, 49%, 99.5%ee). [Column: CHIRALPAK AD (50 mmID × 500 mmL); column temperature, 30 °C; mobile phase, hexane:EtOH = 9:1; flow rate, 80 mL/min; UV detection at 220 nm].

compound **2m:** Mp 165–167 °C. $[\alpha]_D$ = −15.3° (*c* 0.515, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, s), 1.31 (3H, s), 1.32 (3H, d, *J* = 6.6 Hz), 1.81 (1H, br s), 4.06–4.16 (1H, m), 4.33–4.48 (1H, m), 7.39–7.50 (1H, m), 7.61–7.73 (2H, m). Anal. Calcd for $C_{14}H_{15}ClN_2O_2$: C, 60.33; H, 5.42; N, 10.05. Found: C, 60.31; H, 5.72; N, 9.88.

compound **2m'**: Mp 162–164°C. $[\alpha]_D$ = +21.7° (*c* 0.655, $CHCl_3$). Anal. Calcd for $C_{14}H_{15}ClN_2O_2$: C, 60.33; H, 5.42; N, 10.05. Found: C, 60.54; H, 5.63; N, 9.80.

(*S*)-2-{(3-Chlorophenyl)amino}propan-1-ol (18). A flask was charged with K_3PO_4 (66.4 g, 297 mmol), CuI (1.42 g, 7.43 mmol), (*S*)-2-Aminopropan-1-ol (**17**) (11.7 g, 149 mmol), 1-chloro-3-iodobenzene (36.1 g, 149 mmol), ethyleneglycol (16.7 mL, 297 mmol), and i-PrOH

(155 mL) at room temperature. This flask was evacuated and backfilled with argon three times. After stirring at 80 °C for 1 day, the reaction was quenched with water (500 mL) at room temperature. The resulting mixture was extracted with Et_2O twice. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (EtOAc in *n*-hexane) to give **18** (26.9 g, 145 mmol, 97%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (3H, d, *J* = 6.0 Hz), 1.78 (1H, brs), 3.47–3.68 (3H, m), 3.72 (1H, brd, *J* = 10.0 Hz), 6.51 (1H, ddd, *J* = 8.1, 2.3, 0.8 Hz), 6.63 (1H, dd, *J* = 2.3, 2.1 Hz), 6.68 (1H, ddd, *J* = 7.9, 2.1, 0.8 Hz), 7.07 (1H, dd, *J* = 8.1, 7.9 Hz). MS (*m/z*): 186 $(M + H)^+$, 188 $(M +2+ H)^+$.

(*S*)-*N*-[1-{(*tert*-Butyldimethylsilyl)oxy}propan-2-yl]-3-chloroaniline (19). To a solution of **18** (26.9 g, 145 mmol) in DMF (80 mL) were added imidazole (12.8 g, 188 mmol), and TBSCl (29.0 g, 188 mmol) at room temperature. After stirring at 50 °C for 14 h, the reaction was quenched with 1 M aqueous HCl (300 mL, 300 mmol) at room temperature. The resulting mixture was extracted with EtOAc. The organic layer was washed with 1 M aqueous HCl, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 1:0 to 19:1) to give **19** (42.2 g, 141 mmol, 97%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.04 (3H, s), 0.05 (3H, s), 0.90 (9H, s), 1.19 (3H, d, J = 6.2 Hz), 3.46–3.57 (1H, m), 3.61 (2H, d, *J* = 4.2 Hz), 3.90 (1H, d, *J* = 7.4 Hz), 6.46 (1H, d, *J* = 8.1 Hz), 6.58 (1H, s), 6.63 (1H, d, *J* = 7.9 Hz), 7.05 (1H, dd, *J* = 8.1, 7.9 Hz). MS (*m/z*): 300 $(M + H)^+$, 302 $(M +2+ H)^+$.

(*S*)-*tert*-Butyl (3-chlorophenyl)(1-hydroxypropan-2-yl)carbamate (20). To a solution of **19** (42.2 g, 141 mmol) in THF (300 mL) was added 1.6 M *n*-butyllithium solution in *n*-hexane (106

mL, 169 mmol) at −78 °C. After stirring at −78 °C for 0.5 h, a solution of Boc_2O (39.9 g, 183 mmol) in THF (100 mL) was added dropwise to the mixture. The resulting mixture was stirred at −78 °C for 0.5 h under nitrogen atmosphere. Then, the reaction mixture was spontaneously warmed to room temperature, and stirred for 6 h under nitrogen atmosphere. The reaction was quenched with saturated aqueous NH_4Cl (300 mL) at room temperature. The resulting mixture was extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo to give crude product. A solution of this crude product in THF (400 mL) was added 1.0 M TBAF solution in THF (183 mL, 183 mmol) at room temperature. After stirring at room temperature for 20 h under nitrogen atmosphere, the reaction was quenched with water (300 mL) at room temperature. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 1:0 to 9:1) to give **20** (25.2 g, 73.3 mmol, 62%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (3H, d, *J* = 7.2 Hz), 1.37 (9H, s), 2.42 (1H, br s), 3.46–3.59 (1H, m), 3.62–3.74 (1H, m), 4.29–4.45 (1H, m), 7.04–7.08 (1H, m), 7.16–7.19 (1H, m), 7.26–7.30 (2H, m).

(*S*)-*tert*-Butyl (3-chlorophenyl)(1-oxopropan-2-yl)carbamate (21). To a solution of oxalyl chloride (318 mg, 2.45 mmol) in CH_2Cl_2 (6 mL) was added a mixture of DMSO (258 mg, 3.27 mmol) in CH_2Cl_2 (6 mL) at −65 °C. The resulting mixture was stirred at −65 °C to −55 °C for 10 min under nitrogen atmosphere. Then, a solution of **20** (500 mg, 1.64 mmol) in CH_2Cl_2 (8 mL) was added to the mixture, and the mixture was stirred at −65 °C to −55 °C for 10 min under nitrogen atmosphere. Et_3N (0.93 mL, 6.54 mmol) was added to the mixture. After stirring at −65 °C to −55 °C for 30 min under nitrogen atmosphere., the reaction was quenched

with water (30 mL) at −65 °C to −55 °C. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **21** (480 mg, 1.59 mmol, 96%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38–1.44 (3H, m), 1.42 (9H, s), 4.09–4.20 (1H, m), 7.09–7.16 (1H, m), 7.22–7.34 (3H, m), 9.75 (1H, s).

(3*R*,4*S*)-Ethyl 4-{(*tert*-butoxycarbonyl)(3-chlorophenyl)amino}-2,2-difluoro-3-hydroxypentanoate (22a) and (3*S*,4*S*)-ethyl 4-{(*tert*-butoxycarbonyl)(3-chlorophenyl)amino}-2,2-difluoro-3-hydroxypentanoate (22b). To a suspension of Zn (13.6 g, 206 mmol) in THF (25 mL) was added a mixture of **21** (19.5 g, 68.7 mmol) and ethyl 2-bromo-2,2-difluoroacetate (41.3 g, 199 mmol) in THF (110 mL) at room temperature. After stirring under reflux for 1 h under nitrogen atmosphere, the reaction was quenched with a solution of $KHSO_4$ (42.1 g, 300 mmol) in water (300 mL) at 0 °C. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (EtOAc in *n*-hexane) to give a mixture of **22a** and **22b** (16.2 g, 39.6 mmol, 58%). This mixture was used in the next reaction without further purification.

Ethyl 2-{(4*S*,5*R*)-3-(3-chlorophenyl)-4-methyl-2-oxooxazolidin-5-yl}-2,2-difluoroacetate (23) and (4*S*,5*S*)-1-(3-chlorophenyl)-3,3-difluoro-4-hydroxy-5-methylpyrrolidin-2-one (24). A mixture of **22a**, **22b** (16.2 g, 39.6 mmol) and 4 M HCl solution in hexane (100 mL, 400 mmol) was stirred at room temperature for 2 h. Then, the reaction mixture was concentrated in vacuo. The residue was dissolved in THF (275 mL). DIEA (21.3 mL, 119 mmol) was added to the mixture at room temperature. After refluxing for 12 h, water (200 mL) was added to the

mixture at room temperature. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (EtOAc in *n*-hexane) to give **23** (8.10 g, 26.1 mmol, 38% from **21**) and **24** (3.76 g, 14.4 mmol, 21% from **21**). **23**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (3H, t, *J* = 7.2 Hz), 1.46 (3H, d, *J* = 6.0 Hz), 4.39 (2H, q, *J* = 7.2 Hz), 4.60–4.68 (2H, m), 7.18–7.24 (1H, m), 7.33–7.35 (2H, m), 7.48–7.49 (1H, m). **24**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (3H, dd, *J* =6.4, 1.3 Hz), 2.47 (1H, brs), 4.02–4.19 (2H, m), 7.26–7.31 (1H. m). 7.33–7.43 (2H, m), 7.47–7.53 (1H, m).

(4*R*,5*S*)-1-(3-Chlorophenyl)-3,3-difluoro-4-hydroxy-5-methylpyrrolidin-2-one (25). To a solution of **23** (5.37 g, 15.9 mmol) in EtOH (40 mL) were added aqueous 8 M NaOH (6.95 mL, 55.7 mmol) at room temperature. After stirring at 80 °C for 1.5 h, the mixture was neutralized with aqueous 6 M HCl (9.30 mL, 55.7 mmol) at room temperature. Then, the mixture was concentrated in vacuo. The residue was dissolved in MeOH (150 mL). This mixture was refluxed for 4 h. Then, the reaction mixture was concentrated in vacuo. The residue was partitioned between EtOAc and water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using NH silica gel column chromatography (EtOAc in *n*-hexane) to give **25** (4.04 g, 15.4 mmol, 97%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (3H, d, *J* = 6.4 Hz), 2.63 (1H, brs), 4.34–4.61 (2H, m), 7.26–7.33 (1H, m), 7.34–7.43 (2H, m), 7.50 (1H, s).

(4*R*,5*S*)-1-(3-Chloro-4-iodophenyl)-3,3-difluoro-4-hydroxy-5-methylpyrrolidin-2-one (26). To a solution of **25** (3.45 g, 13.2 mmol) in AcOH (22 mL) were added NIS (3.63 g, 15.8 mmol) and concentrated H_2SO_4 (1.43 mL, 26.4 mmol) at room temperature. After stirring at room

temperature for 3 h, water (50 mL) was added to the reaction mixture at room temperature. The resulting mixture was extracted with EtOAc, and the organic layer was washed with saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 4:1 to 0:1) to give **26** (3.82 g, 9.90 mmol, 75%) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (3H, d, *J* = 6.4 Hz), 3.25 (1H, brs), 4.33–4.58 (2H, m), 7.12 (1H, d, *J* = 8.7 Hz), 7.64 (1H, s), 7.90 (1H, d, *J* = 8.7 Hz).

2'-Chloro-4'-{(2*R*,3*S*)-4,4-difluoro-3-hydroxy-2-methyl-5-oxopyrrolidin-1-yl}benzonitrile (2n). To a solution of **26** (3.17 g, 8.19 mmol) in NMP (13 mL) was added CuCN (1.87 g, 20.5 mmol) at room temperature. After stirring at 150 °C for 2.5 h under nitrogen atmosphere, water (100 mL) was added to the reaction mixture at room temperature, and the insoluble portions were filtered off through a pad of Celite®. The filtrate was extracted with EtOAc, and the organic layer was washed with saturated aqueous $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using NH silica gel column chromatography (EtOAc in *n*-hexane), followed by silica gel column chromatography (*n*-hexane:EtOAc = 7:3 to 2:3) to give **2n** (2.02 g, 7.04 mmol, 86%) as a white powder. Furthermore, the recrystallizaion from EtOAc/*n*-hexane gave title compound (1.48 g, 5.65 mmol, 63% from **26**) as colorless prisms. The enantiomeric excess was determined by HPLC analysis (UV: 224 nm, 30 °C); tR1 17.2 min, tR2 18.5 min [Kromasil 5CHI DMB (0.46 cm x 25 cm) (from Eka Chemicals), *n*-hexane:EtOH = 90:10, 1.0 mL/min] to be 99.8% *ee*.[40] Mp: 149–152 °C (EtOAc/n-hexane). $[\alpha]_D$ = +3.2° (*c* 0.500, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (3H, d, *J* = 6.4 Hz), 4.56–4.79 (2H, m), 6.54 (1H, s), 7.87 (1H, dd, *J* = 8.7, 2.1 Hz), 8.08 (1H, d, *J* = 8.7 Hz), 8.15 (1H, d, *J* = 2.1 Hz). Anal. Calcd for $C_{12}H_9N_2O_2ClF_2$: C, 50.28; H, 3.16; N, 9.77. Found: C,

50.21; H, 3.13; N, 9.80.

***N*-(3-Chloro-4-cyanophenyl)-L-leucine (29a).** The mixture of 2-chloro-3-fluorobenznitrile (**28**) (2.00 g, 12.9 mmol), L-leucine (**27a**, 2.02 g, 15.4 mmol) and cesium carbonate (5.45 g, 16.7 mmol) in DMSO (60 mL) was stirred at 90 °C for 12 h. The mixture was extracted with aqueous $NaHCO_3$ twice and aqueous phase was combined. The aqueous phase was acidified with citric acid and extracted with EtOAc twice. The organic layers were combined, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give **29a** (3.43 g, 12.9 mmol, 100%) as a brown oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.96 (3H, d, *J* = 6.0 Hz), 1.01 (3H, d, *J* = 6.2 Hz), 1.65–1.88 (3H, m), 4.06–4.16 (1H, m), 4.50–4.62 (1H, m), 6.50 (1H, dd, *J* = 8.6, 2.4 Hz), 6.65 (1H, d, *J* = 2.5 Hz), 7.42 (1H, d, *J* = 8.5 Hz).

(2*S*)-2-[(3-Chloro-4-cyanophenyl)amino]-3-cyanopropanoic acid (29b). A method similar to that described for **29a** was used to prepare this compound in 72% yield as a brown oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.89–3.10 (2H, m), 4.64–4.76 (1H, m), 6.79 (1H, dd, *J* = 8.7, 2.3 Hz), 6.98 (1H, d, *J* = 2.3 Hz), 7.42 (1H, d, *J* = 8.9 Hz), 7.60 (1H, d, *J* = 8.7 Hz), 13.46 (1H, brs).

2'-Chloro-4'-[(2*S*)-3-hydroxy-2-(2-methylpropyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl]benzo nitrile (30a). To a mixture of **29a** (3.40 g, 12.8 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (2.02 g, 14.0 mmol) and DMAP (2.34 g, 19.1 mmol) in THF (50 mL), CDI (2.48 g, 15.3 mmol) was added portionwise at 0 °C. After stirring at room temperature for 12 h, 5% aqueous $KHSO_4$ was added to the mixture. The mixture was extracted with EtOAc, washed with 5% aqueous $KHSO_4$, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was

dissolved with EtOAc (50 mL) and refluxed for 1 h. The mixture was cooled to room temperature, washed with water, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was washed with IPE/EtOAc to give **30a** (1.18 g, 4.06 mmol, 32%) as a colorless solid. Mp. 163–166 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.67 (3H, d, J = 6.0 Hz), 0.87 (3H, d, J = 6.0 Hz), 1.54–1.75 (3H, m), 5.00 (1H, t, J = 4.1 Hz), 5.03 (1H, s), 7.62 (1H, dd, J = 8.8, 2.0 Hz), 7.93 (1H, d, J = 8.7 Hz), 7.99 (1H, d, J = 2.1 Hz), 12.42 (1H, br s). Anal. Calcd for $C_{15}H_{15}ClN_2O_2$: C, 61.97; H, 5.20; N, 9.64. Found: C, 61.93; H, 5.25; N, 9.42.

2'-Chloro-4'-[(2*S*)-2-(cyanomethyl)-3-hydroxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl]benzonitrile (30b). A method similar to that described for **30a** was used to prepare this compound in 44% yield as a pale yellow solid. Mp. 179–181 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.01–3.27 (2H, m), 5.15 (1H, s), 5.24 (1H, dd, J = 4.9, 3.0 Hz), 7.72 (1H, dd, J = 8.7, 2.3 Hz), 7.96 (1H, d, J = 8.7 Hz), 8.00 (1H, d, J = 2.1 Hz), 12.86 (1H, br s). Anal. Calcd for $C_{13}H_8ClN_3O_2 \cdot 0.1H_2O$+0.1EtOAc: C, 56.61; H, 3.19; N, 14.78. Found: C, 56.59; H, 3.18; N, 14.67.

2'-Chloro-4'-[(2*S*,3*S*)-3-hydroxy-2-(2-methylpropyl)-5-oxopyrrolidin-1-yl]benzonitrile (2o). To a solution of **30a** (550 mg, 1.89 mmol) and acetic acid (1.19 mL, 20.8 mmol) in acetonitrile (20 mL), sodium tetrahydroborate (179 mg, 4.73 mmol) was added portionwise at 0 °C. The mixture was stirred at room temperature for 1 h. Then the mixture was poured into half brine and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 4:1 to 0:1). The residue was recrystallyzed from THF/hexane to give **2o** (342 mg, 1.17 mmol, 62%) as a colorless crystal. Mp. 153–154 °C. $[\alpha]_D$ = −11.5° (*c* 0.500,

$CHCl_3$). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.83 (3H, d, J = 6.6 Hz), 0.94 (3H, d, J = 6.6 Hz), 1.11–1.22 (1H, m), 1.56–1.84 (2H, m), 2.43 (1H, dd, J = 16.9, 3.1 Hz), 2.69–2.80 (1H, m), 4.34–4.45 (2H, m), 5.36 (1H, brs), 7.47 (1H, dd, J = 8.5, 2.1 Hz), 7.75 (1H, d, J = 1.9 Hz), 8.00 (1H, d, J = 8.5 Hz). Anal. Calcd for $C_{15}H_{17}ClN_2O_2$: C, 61.54; H, 5.85; N, 9.57. Found: C, 61.66; H, 5.83; N, 9.51.

2'-Chloro-4'-[(2*S*,3*S*)-2-(cyanomethyl)-3-hydroxy-5-oxopyrrolidin-1-yl]benzonitrile (2p). A method similar to that described for **2o** was used to prepare this compound in 59% yield as a colorless crystal. Mp. 153–156 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.55–2.65 (1H, m), 2.74–2.87 (3H, m), 4.53–4.63 (1H, m), 4.76–4.85 (1H, m), 5.91 (1H, s), 7.74 (1H, dd, J = 8.7, 2.1 Hz), 7.98–8.04 (2H, m). Anal. Calcd for $C_{13}H_{10}ClN_3O_2$: C, 56.64; H, 3.66; N, 15.24. Found: C, 56.57; H, 3.63; N, 15.21.

Gram scale synthesis of compound **2f**.

(4*S*)-4-Ethyl-1,3-oxazolidine-2,5-dione (32). To a suspension of (2*S*)-2-aminobutanoic acid (**31**, 12.5 g、121 mmol) and activated carbon (116 mg) in THF (60 mL) was added dropwise a solution of triphosgene (12.6 g, 42.4 mmol) in THF (60 mL) at room temperature, and the mixture was stirred at 50 °C for 2 h. The same experimental process was performed twice, in each of which insoluble portions were filtered off through a pad of Celite®. The filtrates were combined and concentrated in vacuo. The residue was washed with hexane to give **32** as a solid (27.6 g, 214 mmol, 89%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.06 (3H, t, J = 7.5 Hz), 1.81–2.03 (2H, m), 4.29–4.38 (1H, m), 5.79 (1H, brs).

Benzyl (4*S*)-4-ethyl-2,5-dioxo-1,3-oxazolidine-3-carboxylate (33). A solution of **32** (69.6 g,

539 mmol) and benzyl chloformate (101 g, 593 mmol) in THF (800 mL) was cooled to 0 °C, *N*-methylmorpholine (81.7 g, 808 mmol) was added dropwise, and the mixture was stirred at 0 °C for 2 h. 4 M Hydrogen chloride in EtOAc (86.9 mL) was added dropwise to the reaction mixture at 0 °C, and the precipitated morpholine hydrochloride was filtrated off through a pad of Celite®. The filtrate was concentrated in vacuo to give **33** as a pale yellow oil (91.6 g, 348 mmol, 65%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.95 (3H, t, *J* = 7.5 Hz), 1.75–2.35 (2H, m), 4.74 (1H, dd, *J* = 6.0, 3.2 Hz), 5.27–5.44 (2H, m), 7.28–7.49 (5H, m).

(*S*)-Benzyl 2-ethyl-3-hydroxy-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (34). To a mixture of **32** (91.6 g, 348 mmol) and triethylamine (58.3 mL, 418 mol) in THF (1 L) was added portionwise Meldrum's acid (52.6 g, 365 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. After addition of 5% aqueous potassium hydrogen sulfate, the mixture was extracted with EtOAc. The organic layer was washed with 5% aqueous potassium hydrogen sulfate, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in EtOAc (800 mL), and the mixture was refluxed for 1 h. After addition of 5% aqueous $NaHCO_3$ (800 mL), the aqueous layer was separated and acidified with citric acid monohydride. The mixture was extracted with EtOAc, and the organic layer was dried over $MgSO_4$ and concentrated in vacuo to give **34** as a colorless solid (75.3 g, 288 mmol, 83%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 7.5 Hz), 1.94–2.09 (2H, m), 3.17–3.22 (2H, m), 4.43–4.51 (1H, m), 5.26–5.41 (2H, m), 7.31–7.48 (5H, m).

(2*S*,3*S*)-3-(*tert*-Butyldimethylsilyloxy)-2-ethylpyrrolidin-5-one (4c). To a mixture of **34** (116 g, 444 mmol) and acetic acid (215 mL) in acetonitrile (1.2 L) was added slowly sodium borohydride (30.9 g, 818 mmol) at 0 °C under nitrogen atmosphere, and the mixture was stirred

at room temperature for 18 h. After addition of water, the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in acetonitrile (1.2 L) followed by the addition of acetic acid (215 mL) and sodium borohydride (30.9 g, 818 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The mixture was quenched with water, and was basified with saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by NH column chromatography (EtOAc) to give (2*S*,3*S*)-benzyl 2-ethyl-3-hydroxy-5-oxopyrrolidine-1-carboxylate (117 g, 444 mmol, 100%) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.26 (3H, t, J = 7.2 Hz), 1.75–1.94 (2H, m), 1.76–1.82 (1H, m), 2.61 (1H, dd, J = 17.4, 7.9 Hz), 2.74 (1H, dd, J = 17.4, 7.6 Hz), 4.08–4.23 (1H, m), 4.52–4.64 (1H, m), 5.19–5.35 (2H, m), 7.30–7.47 (5H, m). A mixture of (2*S*,3*S*)-benzyl 2-ethyl-3-hydroxy-5-oxopyrrolidine-1-carboxylate (117 g, 444 mmol), *tert*-butyldimethylsilyl chloride (89.0 g, 589 mmol) and imidazole (47.4 g, 696 mmol) in DMF (2500 mL) was stirred at room temperature for 18 h. After addition of water, the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous NH_4Cl, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in MeOH (800 mL), and 10% Pd/C (50% wet, 10.0 g) was added to the solution. The mixture was stirred under hydrogen atmosphere at room temperature for 6 h. The insoluble portions were filtered off through a pad of Celite®, and the filtrate was concentrated in vacuo. The residue was purified using silica gel column chromatography (*n*-hexane:EtOAc = 50:1 to 1:1) to give **4c** (48.9 g, 201 mmol, 45%) as a colorless oil.

4'-[(2*S*,3*S*)-2-Ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]-2'-(trifluoromethyl)benzonitrile (2f). A mixture of **4c** (6.0 g, 24.7 mmol), 4-iodo-2-(trifluoromethyl)benzonitrile (8.42 g, 28.4 mmol),

Xantphos (2.14 g, 3.70 mmol), $Pd_2(dba)_3$ (1.13 g, 1.23 mmol) and Cs_2CO_3 (12.05 g, 36.98 mmol) in toluene (70 ml) was stirred at 80 ºC for 18 h, and the resulting mixture was partitioned between water and ethylacetate. The organic layer was dried over MgSO4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc = 50:1 to 1:2) to afford 4-((2*S*,3*S*)-3-{[tert-Butyl(dimethyl)silyl]oxy}-2-ethyl-5-oxopyrrolidin-1-yl)-2-(trifluoromethyl) benzonitrile (7.76 g, 18.8 mmol, 76%) as a coloreless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.14 (3H, s), 0.14 (s, 3H), 0.93 (s, 9H), 0.96 (3H, t, *J* = 7.5 Hz), 1,77–1.94 (m, 1H), 2.66 (1H, dd, *J* = 17.0, 6.8 Hz), 2.76 (dd, 1H, *J* = 17.0, 7.2 Hz), 4.16–4.26 (m, 1H), 4.66 (q, *J* = 6.9 Hz), 7.81–7.87 (2H, m), 7.94–7.99 (1H, m). A mixture of 4-((2*S*,3*S*)-3-{[tert-butyl(dimethyl)silyl]oxy}-2-ethyl-5-oxopyrrolidin-1-yl)-2-(trifluoromethyl)benzonitrile (7.76 g, 18.8 mmol) and 6 M HCl (60 ml) in EtOH (30 ml) and THF (60 ml) was stirred at room temperature for 18 h, and partioned between aqueous $NaHCO_3$. and EtOAc. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc = 50:1 to 1:4) to afford **2f** (3.32 g, 11.1 mmol, 59%) as a colorless solid. Furthermore, the recrystallizaion from EtOAc/*n*-hexane gave **2f** (2.36 g, 7.91 mmol, 42%) as a colorless solid. The enantiomeric excess was determined by HPLC analysis (UV: 220 nm, 30 °C); tR1 17.9 min, tR2 24.1 min [CHIRALPAK AD-H (0.46 cm x 25 cm) (from DAICEL), *n*-hexane:EtOH = 90:10, 1.0 mL/min] to be >99.9% *ee*.[42]

Mp. 111–112 °C. $[\alpha]_D = -35.5°$ (*c* 0.588, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, *J* = 7.5 Hz), 1.70–1.86 (3H, m), 2.70 (1H, dd, *J* = 17.4, 4.0 Hz), 2.87 (1H, dd, *J* = 17.4, 6.6 Hz), 4.15–4.24 (1H, m), 4.66–4.75 (1H, m), 7.73 (1H, dd, *J* = 8.5, 2.1 Hz), 7.85 (1H, d, *J* = 8.5 Hz), 7.86 (1H, d, *J* = 2.1 Hz). Anal. Calcd for $C_{14}H_{13}F_3N_2O_2$: C, 56.38; H, 4.39; N, 9.39. Found: C, 56.41; H, 4.35; N, 9.39.

5.2. Biology

5.2.1. AR-binding inhibitory test

To a solution containing a wild-type AR, radiolabeled mibolerone (3 nM) and a compound were added, and the mixture was incubated at 4 °C for 3 h. Bound (B) and free (F) compounds were separated using the dextran/charcoal (DCC) method. The label count of B was measured, and the inhibitory rate of the compound was calculated.

5.2.2. Compound evaluation in the AR reporter assay

Cos-7 cells (at a density of 5×10^6) were sown in a 150-cm^2 flask and grown in a culture medium [DMEM medium containing 10% DCC–fetal bovine serum (FBS) and 2 mM glutamine] for 24 h. Vector DNA containing AR genes and vector DNA containing the luciferase gene bound at the downstream of an androgen-responsive promoter derived from mouse mammary tumor virus (MMTV) were co-transfected using a liposome method. After culturing for 4 h, the cells were harvested, and 10,000 cells were plated in a 96-well plate and cultured for 3 h. In the agonistic assay, DHT or a compound was added, and the cells were further cultured for 24 h, after which the luciferase activity was measured. In the antagonistic assay, DHT (0.1 μM) and a compound were added, and the cells were further cultured for 24 h, after which the luciferase activity was measured. The rate of inhibition by the compound was calculated by setting the luciferase activity induced by the addition of DHT (0.1 μM) as 100.

5.2.3. Animals

Male CD(SD)IGS rats at the age of 4–8 weeks were purchased from Charles River Japan. Female Wistar Imamichi rats at the age of 8 weeks were purchased from CLEA Japan, Inc.

These animals were maintained on a 12-h/12-h light/dark cycle with constant temperature (23 ± 2 °C). Food and water were available ad libitum. Vaginal smears of female rats were examined every morning, and animals that had 4-day regular menstrual cycles were selected for sexual behavior experiments. Bilateral orchidectomies were performed under ether anesthesia.

5.2.4. Reagents

Chemicals and solvents were of reagent grade. The compounds were suspended in 0.5% methylcellulose (Metlose, Shin-Etsu Chemical Co., Ltd., Japan) aqueous solution for oral administration or dissolved in corn oil (Wako Pure Chemical Industries, Ltd., Japan) containing 20% benzyl benzoate (Wako Pure Chemical Industries, Ltd., Japan) for subcutaneous administration. Testosterone propionate (TP) was pharchased from Tokyo chemical industry (Tokyo Japan).

5.2.5. Hershberger assays with immature rats[47]

Male CD(SD)IGS rats at 4 weeks of age were used for assessing the tissue-specific action of compounds. The rats were castrated 5 days before treatment with the compounds started. The compounds were administered twice a day for 4 days consecutively by the oral route. All rats were also treated with 0.5 mg/kg/day of TP. On the next day after the last administration, after sacrificing animals under ether anesthesia, prostates and levator ani muscles were extirpated for weighing.

5.2.6. Hershberger assays with adults rats

Male CD(SD)IGS rats at 8 weeks of age were used for assessing the tissue-specific action

of the compounds. Rats were castrated one day before the treatment with compounds started. The castrated rats were divided into eight different treatment groups (n = 5) : 1) vehicle (0.5% methylcellulose)+0.4 mg/kg/day of testosterone propionate (TP), 2) 0.15 mg/kg/day of compounds+0.4 mg/kg/day of TP, 3) 0.5 mg/kg/day of compounds+0.4 mg/kg/day of TP, 4) 1.5 mg/kg/day of compounds+0.4 mg/kg/day of TP, 5) 5 mg/kg/day of compounds+0.4 mg/kg/day of TP, 6) 15 mg/kg/day of compounds+0.4 mg/kg/day of TP, 7) vehicle+1.2 mg/kg/day of TP. Vehicle or compounds were orally administered once daily. TP was sc administered once daily. The treatment continued for 7 days. On the next day after the last administration the animals were sacrificed by collecting blood from the abdominal aorta under ether anesthesia, and ventral and dorsal prostates with their fluid (both sides) and levator ani muscle were extirpated for weighing.

5.2.7. Sexual behavior tests

Male CD(SD)IGS rats were castrated at the age of 9 weeks, and subcutaneous implantation of a DHEA pellet (1.5 mg, 21-day release; Innovative Research of America, Sarasota, FL) was performed under ether anesthesia at the age of 12 weeks (day0). Treatment with the compounds was started from the next day for 3 weeks. Sexual behavior test were performed on day 18-21 according to the following procedure. A subject male and a 16-weeks old female rat in proestrus that had regular 4-day menstrual cycles were housed in the cage together overnight. Vaginal smears were examined daily for at least 9 days after mating to determine the pseudopregnancy rate.

5.2.8. Metabolic stabilities

Human, mouse, rat, dog and monkey liver microsomes were purchased from Xenotech,

LLC (Lenexa, KS). The reaction mixture was composed of 50 mmol/L phosphate buffer (pH 7.4), 1 mg protein/mL liver microsomes, 1 μmol/L test compound and an NADPH-generating system. The NADPH-generating system in the reaction mixture was composed of 5 mmol/L MgCl2, 5 mmol/L glucose-6-phosphate, 0.5 mmol/L β-NADP+ and 1.5 unit/mL glucose-6-phosphate dehydrogenase. The reaction mixture was incubated at 37°C for 15 min in rats and 60 min in the other species. The reaction was terminated by addition of acetonitrile with an equivalent volume of the reaction mixture. All incubations were made in duplicate. The test compound before and after the incubation was analyzed by UPLC equipped with a PDA detector (Waters, Milford, MA). From the elimination ratio of the compound, metabolic clearance was calculated in the following equation.

Metabolic clearance (μL/min/mg) = Elimination ratio of the compound / Incubation time (min) × microsomal protein concentration (1 mg/mL).

5.2.9. Pharmacokinetic profiles.

Test compound was administered intravenously at a dose of 0.1 mg/kg or orally at a dose of 1 mg/kg to laboratory animals (male, n=3; rats, dogs and monkeys) with fed condition. After administration, blood samples were collected at specified time points and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile followed by centrifugation and the supernatants were analyzed by LC/MS/MS to determine the plasma concentration of the test compound. The PK parameters were calculated by the moment analysis method.

5.2.10. Androgen Receptor molecular biology, protein expression, purification and crystallography.

For each complex, amino acids 671-919 of wild-type human androgen receptor (wtAR) were over-expressed as 6xHis tag protein in E. coli. The wtAR protein was then purified by immobilized Ni^{2+} affinity chromatography, followed by size exclusion with a Superdex 200 column (GE Healthcare), and then concentrated to between 10-20 mg/ml in buffer consisting of 25mM HEPES pH 7.2, 150mM $LiSO_4$, 10mM DTT, 10% glycerol, 0.1% beta-octylglucoside, and 100 μM of inhibitor. It is noteworthy that successful complex structures of wtAR were generated only when inhibitor compound was present at $\geq$ 100 μM from expression through to final concentration of the protein.

Crystals suitable for data collection were obtained by vapor diffusion in sitting drops at 20°C. Reservoirs contained 0.8225M–1.0763M ammonium phosphate dibasic, 0.03M–0.05M potassium phosphate dibasic, and 0.03M–0.05 M sodium phospate monobasic were mixed 50nl:50nl with protein in hanging droplets. Resulting crystals were immersed in mother liquor solution containing 20-30% glycerol for cryo protection and flash frozen in liquid nitrogen. Crystals of wtAR complexes described grew in the orthorhombic space group $P2_12_12_1$ with similar unit cell dimensions and 1 molecule in the asymmetric unit.

Diffraction data were collected from single cryogenically protected crystals at either beamline 5.0.2 or 5.0.3 of the Advanced Light Source at Lawrence Berkeley National Laboratory. Data were reduced using the HKL2000 software package.[53] The structures were determined by molecular replacement with either MOLREP[54] or PHASER[55] of the CCP4 program suite and refined with the program REFMAC.[56] Several cycles of model building with COOT[57] and refinement was performed to improve the quality of each model. Data reduction and refinement statistics are summarized in Table S1 (supplementary data).

The coordinates and structure factors are deposited in Protein Data Bank and assigned the accession codes X (co-crystal of 2f with AR).

Supporting information available: Includes information methods used in molecular modeling, enzyme assays, cell line and animal models, pharmacokinetics, metabolic stability, and structural analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

1) Gao,W.; Bohl, C. E.; Dalton, J. T. Chemistry and Structural Biology of Androgen Receptor. *Chem. Rev.* **2005**, *105*, 3352–3370.

2) Moodradian, A. D.; Morley, J. E.; Korenman, S. G. Biological Actions of Androgens. *Endocrine Reviews* **1987**, *8*, 1–28.

3) Matsumoto, T.; Sakari, M.; Okada, M.; Yokoyama, A.; Takahashi, S.; Kouzmenko, A.; Kato, S. The Androgen Receptor in Health and Disease. *Annu. Rev. Physiol.* **2013**, *75*, 201–224.

4) Fang, H.; Tong, W.; Branham, W. S.; Moland, C. L.; Dial, S. L.; Hong, H.; Xie, Q.; Perkins, R.; Owens, W.; Sheehan, D. M. Study of Natural, Synthetic, and Environmental Chemicals for Binding to the Androgen Receptor. *Chem. Res. Toxicol.* **2003**, *16*, 1338–1358.

5) Miller, W. L. Molecular Biology of Steroid Hormone Synthesis. *Endocr. Rev.* **1988**, *9*, 295–318.

6) Russell, D. W.; Berman, D. M.; Bryant, J. T.; Cala, K. M.; Davis, D. L.; Landrum, C. P.; Prihoda, J. S.; Silver, R. I.; Thigpen, A. E.; Wigley, W. C. The Molecular Genetics of Steroid 5α-Reductases. *Recent Prog. Horm. Res.* **1994**, *49*, 275–284.

7) Roy, A. K.; Chatterjee, B. Androgen Action. *Crit. Rev. Eukaryot. Gene Expr.* **1995**, *5*, 157–176.

8) Pratt, W. B.; Toft, D. O. Steroid Receptor Interactions with Heat Shock Protein and Immunophilin Chaperones. *Endocr. Rev.* **1997**, *18*, 306–360.

9) Ahern, T.; Wu, C. W. New horizons in testosterone and the ageing male. *Age Ageing* **2015**, *44*, 188–195.

10) Isidori, A. M.; Greco, E. A.; Aversa, A. Androgen deficiency and hormone-replacement therapy. *BJU Int.* **2005**, *96*, 212–216.

11) Narayanan, R.; Mohler, M. L.; Bohl, C. E.; Miller, D. D.; Dalton, J. T. Selective androgen receptor modulators in preclinical and clinical development. *Nucl. Recept. Signaling* **2008**, *6*. DOI: 10.1621/nrs.06010

12) Crawford, J. Clinical results in cachexia therapeutics. *J. Curr. Opin. Clin. Nutr. Metab. Care*. **2016**, *19*, 199–204.

13) Hsieh, M. J.; Yang, T. M.; Tsai, Y. H. Nutritional supplementation in patients with chronic obstructive pulmonary disease. *J. Formosan Medical Association*. DOI: 10.1016/j.jfma.2015.10.008

14) Harman, S. M.; Metter, E. J.; Tobin, J. D.; Pearson, J.; Blackman, M. R. Longitudinal Effects of Aging on Serum Total and Free Testosterone Levels in Healthy Men. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 724–731.

15) Peluso, I.; Palmery, M. The relationship between body weight and inflammation: Leson from anti-TNF-α antibody therapy. *Human Immunology*. **2016**, *77*, 47–53.

16) Udediba, E.; Kaminetsky, J. Phase II drugs currently being investigated for the treatment of hypogonadism. *Expert Opin. Investig. Drugs* **2014**, *23*, 1605–1618.

17) Choi, S. M.; Lee, B-M. Comparative safety evaluation of selective androgen receptor modulators and anabolic androgenic steroids. *Expert Opin. Drug Saf.* **2015**, *14*, 1773–1785.

18) Bhasin, S.; Bremner, W. J. Emerging Issues in Androgen Replacement Therapy *J. Clin. Endocrinol. Metab.* **1997**, *82*, 3–8.

19) Sun, C.; Robl, J. A.; Wang, T. C.; Huang, Y.; Kuhns, J. E.; Lupisella, J. A.; Beehler, B. C.; Golla, R.; Sleph, P. G.; Seethala, R.; Fura, A.; Krystek, Jr. S. R.; An, Y.; Malley, M. F.; Sack, J. S.; Salvati, M. E.; Grover, G. J.; Ostrowski, J.; Hamann, L. G. Disconfery of Potent, Orally-Active, and Muscle-Selective Androgen Receptor Modulators Based on an *N*-Aryl-hydroxybicyclohydantoin Scaffold. *J. Med. Chem.* **2006**, *49*, 7596–7599.

20) Gelfand, M. M.; Wiita, B. Androgen and estrogen-androgen hormone replacement therapy: a review of the safety literature, 1941 to 1996. *Clin. Ther*. **1997**, *19*, 383–404.

21) Mohler, M. L.; Bohl, C. E.; Jones, A.; Coss, C. C.; Narayanan, R.; He, Y.; Hwang, D. J.; Dalton, J. T.; Miller, D. D. Nonsteroidal Selective Androgen Receptor Modulators (SARMs): Dissociating the Anabolic and Androgenic Activities of the Androgen Receptor for Therapeutic Benefit. *J. Med. Chem.* **2009**, *52*, 3597–3617.

22) Zhang, X.; Sui, Z. Deciphering the selective androgen receptor modulators paradigm. *Expert Opin. Drug Discov.* **2013**, *8*, 191–218.

23) Haendler, B.; Cleve, A. Recent Developments in antiandrogens and selective androgen receptor modulators. *Moll. Cell Endocrinol*. **2012**, *352*, 79–91.

24) Cosman, F.; Lindsay, R. Selective Estrogen Receptor Modulators: Clinical Spectrum. *Endocr. Rev*. **1999**, *20*, 418–434

25) Jones, A.; Chen, J.; Hwang, D. J.; Miller, D. D.; Dalton, J. T. Preclinical Characterization of a (*S*)-*N*-(4-Cyano-3-Trifluoromethyl-Phenyl)-3-(3-Fluoro,4-Chlorophenoxy)-2-Hydroxy-2-Methyl-Propanamide: A Selective Androgen Receptor Modulator for Hormonal Male Contraception. *Endocrinology* **2009**, *150*, 385–395.

26) Srinath, R.; Dobs, A. Enobosarm (GTx-024, S-22): a potential treatment for cachexia. *Future Oncol.* **2014**, *10*, 187–194.

27) Ostrowski, J.; Kuhns, J. E.; Lupisella, J. A.; Manfredi, M. C.; Beehler, B. C.; Krystek, S. R., Jr.; Bi, Y.; Sun, C.; Seethala, R.; Golla, R.; Sleph, P. G.; Fura, A.; An, Y.; Kish, K. F.; Sack, J. S.; Mookhtiar, K. A.; Grover, G. J.; Hamann, L. G. Pharmacological and X-Ray Structural Characterization of a Novel Selective Androgen Receptor Modulatior: Potent

Hyperanabolic Stimulation of Skeletal Muscle with Hypostimulation of Prostate in Rats. *Endocrinology* **2007**, *148*, 4–12.

28) Martinborough, E.; Shen, Y.; Oeveren, A.; Long, Y. O.; Lau, T. L.; Marschke, K. B.; Chang, W. Y.; Lopez, F. J.; Vajda, E. G.; Rix, P. J.; Viveros, O. H.; Negro-Vilar, A.; Zhi, L. Substituted 6-(1-Pyrrolidine)quinolin-2(1*H*)-ones as Novel Selective Androgen Receptor Modulators. *J. Med. Chem.* **2007**, *50*, 5049–5052.

29) Basaria, S.; Collins, L.; Dillon, E. L.; Orwoll, K.; Storer, T. W.; Miciek, R.; Uloor, J.; Zhang, A.; Eder, R.; Zientek, H.; Gordon, G.; Kazmi, S.; Sheffield-Moore, M.; Bhasin, S. The safety, pharmacokinetics, and effects of LGD-4033, a novel nonsteroidal oral, selective androgen receptor modulator, in healthy young men. *J. Gerontol A. Biol. Sci. Med. Sci.* **2013**, *68*, 87–95.

30) Turnbull, P.; Cadilla, R.; Shen, Y.; Poole, C.; Stewart, E.; Gampe, R.; Clark, R.; Henke, B.; Russell, A. Discovery of GSK2881078A: A selective androgen receptor modulator (SARM) for the treatment of muscle wasting disorders. Abstracts of Papers, 249th ACS National Meeting & Exposition, Denver, CO, United States, Mar 22-26, 2015; MEDI 247.

31) Narayanan, R.; Ahn, S.; Cheney, M. D.; Yepuru, M.; Miller, D. D.; Steiner, M. S.; Dalton, J. T. Selective Androgen Receptor Modulators (SARMs) Negatively Regulate Triple-Negative Breast Cancer Growth and Epithelial: Mesenchymal Stem Cell Signaling. *PLoS ONE* **2014**, *9*, 7 e103202.

32) Nique, F. Hebbe, S.; Triballeau, N.; Peixoto, C.; Lefrançois, J.-M.; Jary, H.; Alvey, L.; Manioc, M.; Housseman, C.; Klaassen, H.; Beeck, K. V.; GuééD.; Namour, F.; Minet, D.; Van der Aar, E.; Feyen, J.; Fletcher, S.; Blanqué, R.; Robin-Jagerschmidt, C.; Deprez, P. Identification of a 4-(Hydroxymethyl)diarylhydantoin as a Selective Androgen Receptor Modulator. *J. Med. Chem.* **2012**, *55*, 8236-8247.

33) Cozzoli, A.; Capogrosso, R. F.; Sblendorion, V. T.; Dinardo, M. M.; Jagerschmidt, C.; Namour, F.; Camerino, G. M.; Luca, A. D. GLPG0492, a novel selective androgen receptor modulator, improves muscle performance in the exercised-mdx mouse model of muscular dystrophy. *Pharm. Res.* **2013**, *72*, 9-24.

34) Asano, M.; Hitaka, T.; Imada, T.; Yamada, M.; Megumi, M.; Shinohara, H.; Hara, T.; Yamaoka, M.; Santou, T.; Nakayama, M.; Imai, Y.; Habuka, N.; Yano, J.; Wilson, K.; Fujita, H.; Hasuoka, A. Synthesis and biological evaluation of novel selective androgen receptor modulators (SARMs). Part II: Optimization of 4-(pyrrolidin-1-yl)benzonitrile derivatives. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1897-1901.

35) Supplementary Table 2 in supporting information.

36) The optical purity of compound **5** was confirmed in reference 34.

37) Supporting information (S3)

38) Supporting information (S6)

39) The activity **2m'**; IC_{50} = 6600 nM, EC_{50} < 10000 nM.

40) Supporting information (S4)

41) The analytical condition was depicted in experimental section for **2n**.

42) Synthesis of ***rac*-2n** is depicted in supporting information (S1).

43) Supporting information (S5)

44) The analytical condition was depicted in experimental section for **2f**.

45) Synthesis of ***rac*-2f** is depicted in supporting information (S2).

46) Aikawa, K.; Miyawaki, T.; Hitaka, T.; Imai, Y.; Hara, T.; Miyazaki, J.; Yamaoka, M.; Kusaka, M.; Kanzaki, N.; Tasaka, A.; Shiraish, M.; Yamamoto, S. Synthesis and biological evaluation of novel selective androgen receptor modulators (SARMs). Part I. *Bioorg. Med. Chem.* **2015**, *23*, 2568–2578.

47) Hershberger, L. G.; Shipley, E. G.; Meyer, R. K. Myotrophic Activity of 19-Nortestosterone and Other Steroids Determined by Modified Levator Ani Muscle Method.*Proc. Soc. Exp. Biol. Med*. **1953**, *83*, 175–180.

48) Supplementary Table 3

49) Hikichi, Y.; Yamaoka, M.; Kusaka, M.; Hara, T. Selective androgen receptor modulator activity of a steroidal antiandrogen TSAA-291 and its cofactor recruitment profile. *Eur. J. Pharmacol.* **2015**, *765*, 322–331.

50) Meijsing, S. H.; Pufall, M. A.; So, A. Y; Bates, D. L.; Chen, L; Yamamoto, K. R. DNA Binding Site Sequence Directs Glucocorticoid Receptor Structure and Activity. *Science.* **2009**, *324*, 407–410.

51) Saeed, A.; Vaught, G, M.; Gavardinas, K.; Matthews, D.; Green, J, E.; Losada, P. G.; Bullock, H. A.; Calvert, N. A.; Patel, Nita. J.; Sweetana, S. A.; Krishnan, Ve.; Henck, J. W.; Luz, J. G.; Wang, Y.; Jadhav, Prabhakar. 2-Chloro-4-[[(1*R*,2*R*)-2-hydroxy-2-methyl-cyclopentyl]amino]-3-methyl-benzonitrile: A Transdermal Selective Androgen Receptor Modulator (SARM) for Muscle Atrophy. *J. Med. Chem.* **2016**, *59*, 750–755

52) Ullrich, T.; Sasmal, S.; Boorgu, V.; Pasagadi, S.; Cheera, S.; Rajagopalan, S.; Bhumireddy, A.;Shashikumar, D.; Chelur, S.; Belliappa, C.; Pandit, C.; Krishnamurthy, N.; Mukherjee, S.; Ramanathan, A.; Ghadiyaram, C.; Ramachandra, M.; Santos, P. G.; Lagu, B.; Bock, M. G.; Perrone, M. H.; Weiler, S.;Keller, H. 3-Alkoxy-pyrrolo[1,2-*b*]pyrazolines as selective androgen receptor modulators with idea physiochemical properties for transdermal administration. *J. Med. Chem.* **2014**, *57*, 7396– 7411.

53) Otwingowski, Z.; Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **1997**, *276*, 307-326.

54) Vagin, A.; Teplyakov, A. MOLREP: an automated program from molecular replacement. *J. Appl. Crystallogr.* **1997**, *30*, 1022-1025.

55) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser crystallographic software. *J. Appl. Cryst.* **2007**, *40*, 658-674.

56) Murshudov, G. N.; Vagin, A. A.; Dodson, E. Refinement of macromolecular structures by the maximum-likelihood method. *J. Acta Crystallogr., Sect. D* **1997**, *53*, 240-255.

57) McRee, D. E. XtalView/Xfit-a versatile program for manipulating atomic coordinates and electron density. *J. Struct. Biol.* **1999**, *125*, 156-165.

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SUPPORTING INFORMATION

Synthesis and biological evaluation of novel Selective Androgen Receptor Modulators (SARMs): Part III

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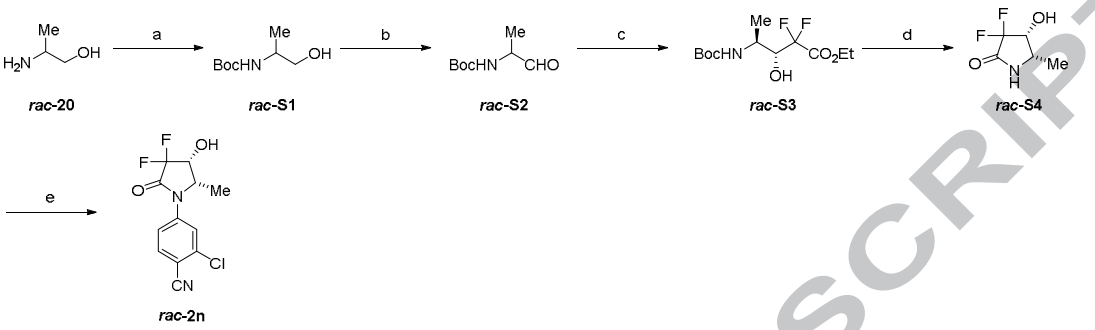
S1 Synthesis of ***rac*-2n** for confirming the optical purity of **2n**

Scheme S1. Synthesis of ***rac*-2n**.



[a]Reagents and conditions: (a) Boc_2O, 1 M aqueous NaOH, 96%; (b) $SO_2 \cdot Py$ (19.7 g, 111 mmol), DMASO, 56%; (c) $Br(F)_2CCO_2Et$, Zn, THF, reflux, 60%; (d) (1) 4 M HCl in EtOAc; (2) *i*-Pr_2NEt, THF, reflux, 68% for 2 steps; (a) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, 18%.

rac-tert*-Butyl (1-hydroxypropan-2-yl)carbamate (*rac*-S1).** To a solution of -2-Aminopropan-1-ol (rac*-20**) (3.00 g, 39.1 mmol) in THF (60 mL) were added a solution of 1 M aqueous NaOH (30 mL, 30.0 mmol) and a solution of Boc_2O (10.3 g, 47.0 mol) in THF (20 mL) at room temperature. After stirring at room temperature for 14 h, the reaction mixture was extracted with Et_2O twice. The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (EtOAc in *n*-hexane) to give ***rac*-S1** (6.61 g, 37.6 mmol, 96%) as a white powder. Mp. 45–50 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (3H, d, *J* = 6.8 Hz), 1.45 (9H, s), 3.09 (1H, br s), 3.50 (1H, dd, *J* = 10.9, 6.0 Hz), 3.62 (1H, dd, *J* = 10.9, 3.8 Hz), 3.67–3.83 (1H, m), 4.81 (1H, brd, *J* = 6.6 Hz). Anal. Calcd for $C_8H_{17}NO_3$: C, 54.84; H, 9.78; N, 7.99. Found: C, 54.83; H, 9.82; N, 7.74.

***rac-tert*-Butyl (1-oxopropan-2-yl)carbamate (*rac*-S2).** To a solution of *rac-tert*-Butyl

(1-hydroxypropan-2-yl)carbamate (***rac*-S1**) (6.50 g, 37.1 mmol) in DMSO (65 mL) was added a solution of SO_2•Py (19.7 g, 111 mmol) in DMASO (65 mL) at 0 °C (ice bath). After stirring at 0 °C for 2 h under nitrogen atmosphere, the reaction was quenched with aqueous 1 M citric acid (130 mL) at room temperature. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (hexane:EtOAc = 1:0 to 7:3) to give ***rac*-S2** (3.62 g, 12.2 mmol, 56%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (3H, d, *J* =7.4 Hz), 1.46 (9H, s), 4.16–4.35 (1H, m), 5.12 (1H, br s), 9.56 (1H, br s).

***rac*-(3R*,4S*)-*tert*-Ethyl 4-{(*tert*-butoxycarbonyl)amino}-2,2-difluoro-3-hydroxypentanoate (*rac*-S3).** To a suspension of Zn (4.01 g, 60.6 mmol) in THF (8 mL) was added a mixture of ***rac*-2** (3.50 g, 20.2 mmol) and ethyl 2-bromo-2,2-difluoroacetate (12.1 g, 58.6 mmol) in THF (35 mL) at room temperature. After stirring under reflux for 1 h under nitrogen atmosphere, the reaction was quenched with 1 M aqueous $KHSO_4$ (65 mL) at room temperature. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified using silica gel column chromatography (EtOAc) to give title compound (3.62 g, 12.2 mmol, 60%) as yellow oil, which was used in the next reaction without further purification.

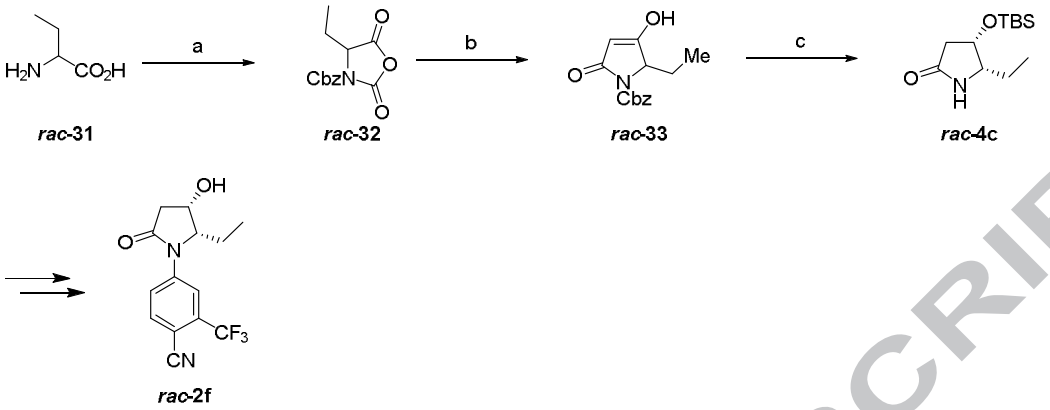
***rac*-(3R*,2S*)-4,4-Difluoro-3-hydroxy-2-methylpyrrolidin-5-one (*rac*-S4).** A mixture of ***rac*-S3** (3.62 g, 12.2 mmol) and 4 M HCl solution in EtOAc (20 mL, 80.0 mmol) was stirred at room temperature for 2 h. Then, the mixture was concentrated in vacuo. The residue was dissolved in THF (60 mL). To the resulting mixture was added DIEA (6.30 mL, 36.5 mmol) at room temperature. After stirring under reflux for 3 h, the reaction mixture was concentrated in

vacuo. The residue was passed through short silica gel column chromatography (EtOAc). The filtrated was concentrated in vacuo. The residue was washed with IPE to give ***rac*-S4** (1.26 g, 8.34 mmol, 68%) as a pale yellow powder. Mp. 134–138. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.06 (3H, dd, J = 6.6, 0.9 Hz), 3.71 (1H, dt, J = 13.0, 6.6 Hz), 4.18–4.33 (1H, m), 6.12 (1H, d, J = 5.9 Hz), 8.85 (1H, br s). MS (API): m/z 152 $(M + H)^+$; Anal. Calcd for $C_5H_7NO_2F_2$: C, 39.74; H, 4.67; N, 9.27. Found: C, 39.84; H, 4.65; N, 9.14.

***rac*-2-Chloro-4-{(*3R*,2S**)-4,4-difluoro-3-hydroxy-2-methyl-5-oxopyrrolidin-1-yl}benzonitrile (*rac*-2n)**

A test tube was charged with 4-bromo-2-chlorobenzonitrile (1.24 g, 5.51 mmol), ***rac*-S4** (1.00 g, 6.62 mmol), Xantphos (488 mg, 827 μmol), $Pd_2(dba)_3$ (252 mg, 276 μmol), Cs_2CO_3 (2.65 g, 7.72 mmol), and 1,4-dioxane (6 mL) at room temperature. The resulting test tube was evacuated and backfilled with argon three times. After being stirred at 100 °C for 3 h under microwave irradiated conditions, the reaction mixture was passed through short silica gel column chromatography (EtOAc). The filtrated was concentrated in vacuo. The residue was purified using silica gel column chromatography (hexane:EtOAc = 9:1 to 2:3) and NH silica gel column chromatography (hexane:EtOAc = 7:3 to 3:7), followed by the recrystallization from EtOAc/n-hexane to give ***rac*-2n** (286 mg, 1.00 mmol, 18%) as a pale yellow powder. Chiral HPLC analysis showed racemic mixture (UV: 224 nm, 30 °C); tR1 17.2 min, tR2 18.5 min [Kromasil 5CHI DMB (0.46 cm x 25 cm) (from Eka Chemicals), *n*-hexane/EtOH = 90:10, 1.0 mL/min]. Mp: 136–139 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.16 (3H, d, J = 6.2 Hz), 4.51–4.80 (2H, m), 6.54 (1H, s), 7.87 (1H, d, J = 8.5 Hz), 8.08 (1H, d, J = 8.5 Hz), 8.15 (1H, s).

S2 Synthesis of ***rac*-2f** for confirming the optical purity of **2f**



A method similar to that described for **2f** was used to prepare ***rac*-2f** using ***rac*-31** as the starting compound. The ^{1}H NMR and MS spectrum data of rac**-2f** was identical to that of **2f**.

Compound ***rac*-2f**: Mp. 94–95 °C. $[\alpha]_D$ = +1.2° (*c* 0.498, $CHCl_3$). Anal. Calcd for $C_{14}H_{13}F_3N_2O_2$: C, 56.38; H, 4.39; N, 9.39. Found: C, 56.46; H, 4.37; N, 9.37.

S3 Determination of relative structure of **2d** by X-ray information

Crystal data for **2d**: $C_{13}H_{13}ClN_2O_2$, *MW* = 264.71; crystal size, 0.12 x 0.06 x 0.05 mm; colorless, block; monoclinic, space group *C*2, *a* = 33.5164(16) Å, *b* = 4.6779(3) Å, *c* = 16.6217(9) Å, $\alpha = \gamma = 90°$, β = 103.039(7)°, *V* = 2538.8(2) Å^3, *Z* = 8, *Dx* = 1.385 g/cm^3, *T* = 100 K, μ = 2.639 mm^{-1}, λ = 1.54187 Å, R_1 = 0.052, wR_2 = 0.104, Flack Parameter[S1] = 0.038(6).

All measurements were made on a Rigaku R-AXIS RAPID-191R diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SIR2008[S2] and was refined using full-matrix least-squares on F^2 with SHELXL-2013.[S3] All non-H atoms were refined with anisotropic displacement parameters.

CCDC 1526941 for compound **2d** contains the supplementary crystallographic data for this

paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?.

Drawing Structure

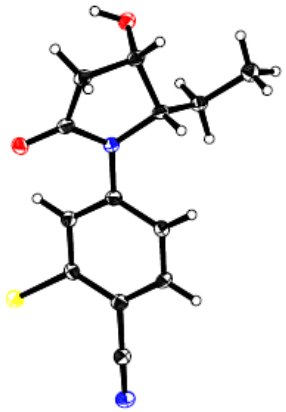


Figure S1. ORTEP of **2d**, thermal ellipsoids are drawn at 30% probability.

S4 Determination of relative structure of **2n** by X-ray information

Crystal data for **2n**: $C_{12}H_9ClF_2N_2O_2$, *MW* = 286.67; crystal size, 0.39 x 0.36 x 0.27 mm; colorless, block; orthorhombic, space group $P2_12_12_1$, a = 7.5374(4) Å, b = 11.0456(5) Å, c = 14.0831(8) Å, $\alpha = \beta = \gamma = 90°$, V = 1172.50(10) Å^3, Z = 4, Dx = 1.624 g/cm^3, T = 100 K, μ = 3.166 mm^{-1}, λ = 1.54187 Å, R_1 = 0.049, wR_2 = 0.120, Flack Parameter[S1] = 0.006(5).

All measurements were made on a Rigaku R-AXIS RAPID-191R diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SIR2008[S2] and was refined using full-matrix least-squares on F^2 with SHELXL-2013.[S3] All

non-H atoms were refined with anisotropic displacement parameters.

CCDC 1526940 for compound **2n** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?.

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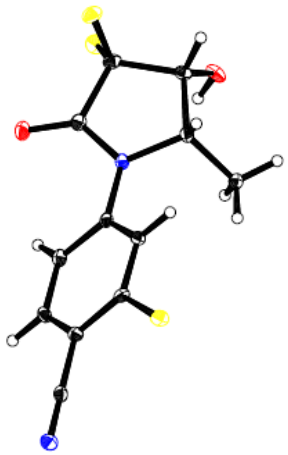


Figure S2. ORTEP of **2n**, thermal ellipsoids are drawn at 30% probability.

S5 Determination of relative structure of **2o** by X-ray information

Crystal data for **2o**: $C_{15}H_{17}ClN_2O_2$, *MW* = 292.76; crystal size, 0.45 x 0.26 x 0.06 mm; colourless, plate; orthorhombic, space group $P2_12_12_1$, a = 5.95380(6) Å, b = 10.43270(10) Å, c = 24.0656(3) Å, $\alpha = \beta = \gamma = 90°$, V = 1494.82(3) Å^3, Z = 4, Dx = 1.301 g/cm^3, T = 100 K, μ = 2.290 mm^{-1}, λ = 1.54187 Å, R_1 = 0.031, wR_2 = 0.076, Flack Parameter[S1] = 0.005(8).

All measurements were made on a Rigaku XtaLAB P200 diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SIR2008[S2] and was refined using full-matrix least-squares on F^2 with SHELXL-2014/7.[S3] All non-H atoms were refined with anisotropic displacement parameters.

CCDC 1526942 for compound **2o** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?.

Drawing Structure

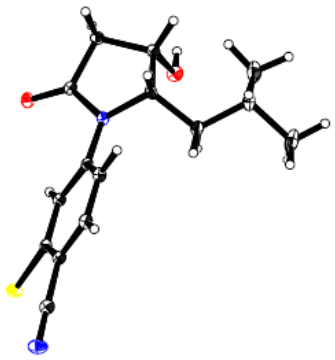
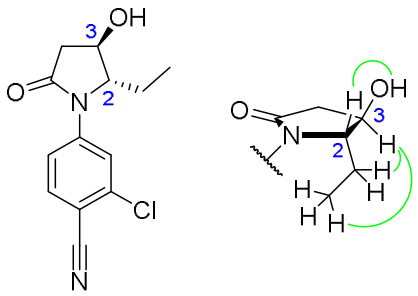


Figure S3. ORTEP of **2o**, thermal ellipsoids are drawn at 30% probability.

S6 Relative configuration of **2e,i,m** by NMR analysis.

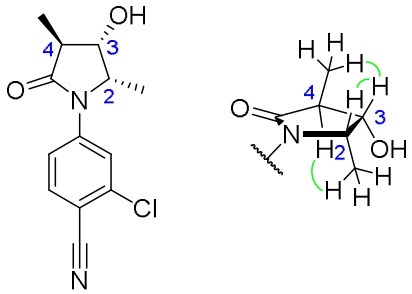
2'-Chloro-4'-[(2*S*,3*R*)-2-ethyl-3-hydroxy-5-oxopyrrolidin-1-yl]benzonitrile (**2e**)

^{1}H-^{1}H NOESY (600 MHz, DMSO-d_6): H2/OH; H3/CH$_2$-CH$_3$.



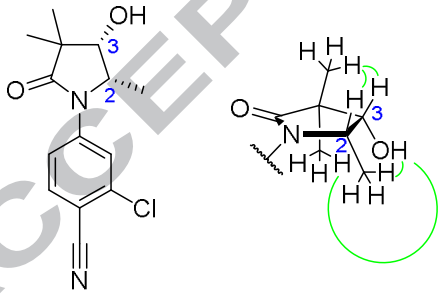
2'-Chloro-4'-[(2*S*,3*S*,4*S*)-3-hydroxy-2,4-dimethyl-5-oxopyrrolidin-1-yl]benzonitrile (**2i**)

^{1}H-^{1}H NOESY (600 MHz, CD_3CN): H3/H2, C4-C$\underline{H}_3$; H4/C2-C$\underline{H}_3$.



2'-Chloro-4'-[(2*S*,3*S*)-3-hydroxy-2,4,4-trimethyl-5-oxopyrrolidin-1-yl]benzonitrile (**2m**)

^{1}H-^{1}H NOESY (600 MHz, DMSO-d_6): CC$\underline{H}_3$CH$_3$/H3, H2; O$\underline{H}$/CCH$_3$C$\underline{H}_3$, C2-C$\underline{H}_3$.



S7 Supplementary Table 1: Data collection and Refinement Statistics

Crystal	AR_WT / **2f**

Crystal ID	168106c8A
Data collection	
Beamline	5.0.3 (ALS)
Space group	$P2_12_12_1$
Unit cell dimensions	
a, b, c (Å)	54.7, 65.6, 70.9
, , (°)	90, 90, 90
Resolution (Å)	40 - 1.44 (1.46–1.44)
Observed reflections	88987
Unique reflections	47137
Redundancy	4.6 (4.0)
Completeness (%)	99.6 (97.0)
I/	30.1 (2.0)
R_{sym} [a]	0.040 (0.652)
R_{meas} [b]	0.051
R_{pim} [b]	0.020
Molecules in ASU	1
Refinement	

Resolution (Å)	40–1.44 (1.48–1.44)
Reflections	44486
R_{work} [b]	0.193 (0.273)
R_{free} [b]	0.217 (0.284)
Number of atoms	
Protein	2068
Ligand/Ion	28
Water	159
Glycerol (molecules)	3
Average B factor (Å^2) [d]	16.7
Rms deviation from ideal geometry	
bond lengths (Å)	0.005
bond angles (°)	0.95
Ramachandran plot (%) [e]	
Preferred regions	99.2
Allowed regions	0.8
Outliers	0.0
PDB code	5V8Q

[a] $R_{sym}=\Sigma_h\Sigma_i|I(h)_i-<I(h)>|/\Sigma_h\Sigma_i<I(h)>$, where <I(h)> is the mean intensity of symmetry-related reflections. [b] R_{meas} (=R_{rim}) and R_{pim} were calculated with SCALA in the CCP4 program suite. $R_{meas}=\Sigma_h[N/(N-1)]^{1/2}\Sigma_i|I(h)_i-<I(h)>|/\Sigma_h\Sigma_i<I(h)>$, $R_{pim}=\Sigma_h[1/(N-1)]^{1/2}\Sigma_i|I(h)_i-<I(h)>|/\Sigma_h\Sigma_i<I(h)>$. [c] $R_{work}=\Sigma||F_{obs}|-|F_{calc}||/\Sigma|F_{obs}|$. R_{free} was calculated for randomly chosen 5% of reflections excluded from refinement. [d] B-factor includes contributions from TLS parameters. [e] Calculated with Coot. Values in parentheses are for the highest resolution shell.

S8 Supplementary Table 2 : Metabolic stabilities

All pyrolidone derivatives except for 2k showed excellect metabolic stabilities.

Compd.	Metabolic stabilities (μL/min/mg)	
	Human	Rat
2a	6	13
2b	0	-10
2c	3	8
2d	2	24
2e	-3	1
2f	-1	20
2g	2	20
2h	-15	0
2i	2	4
2j	5	32
2k	152	220
2l	2	6

2m	1	8
2n	-3	-6
2o	-19	41
2p	N.D.*	N.D.*

*N.D.; Not determined.

S9 Supplementary Table 2 : PK profiles of compound **2b** (Crl:CD(SD) rat, male).

p.o. (1 mg/kg)			
C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}24h}$ (ngh/mL)	MRT (h)
1073.6	3.33	7336.9	4.06

[a]Data are expressed as the mean ± S.D. (n = 3).

S10 References

S1) Flack, H. D. Acta Cryst. A 1983, 39, 876–881.

S2) M.C. Burla, R. Caliandro, M. Camalli, B. Carrozzini, G.L. Cascarano, L. De Caro, C. Giacovazzo, G. Polidori, D. Siliqi, R. Spagna, SIR2008: Program for the Solution of Crystal Structures from X-ray Data, CNR Institute of Crystallography, Bari, Italy, 2007.

S3) Sheldrick, G.M. Acta Cryst. A 2008, 64, 112–122.

Graphical Abstract

Synthesis and biological evaluation of novel Selective Androgen Receptor Modulators (SARMs) Part III: Discovery of 4-(5-oxopyrrolidine-1-yl) benzonitrile derivative 2f as a clinical candidate

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