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(D)-2-*tert*-Butoxycarbonylamino-5,5-difluoro-5-phenyl-pentanoic acid: Synthesis and incorporation into the growth hormone secretagogues

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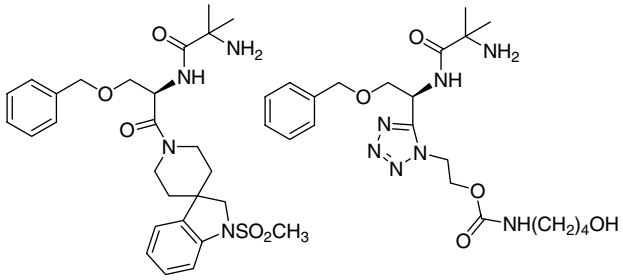
ABSTRACT

The first enantioselective synthesis of (D)-2-*tert*-butoxycarbonylamino-5,5-difluoro-5-phenyl-pentanoic acid **3** was achieved. The incorporation of the titled compound into growth hormone secretagogue (GHS) compounds resulted in new analogs **10** and **16**, both of which had significantly increased in vitro potency. The compound **10** also showed improved in vivo efficacy as well as pharmacokinetic properties in rat models.

The discovery of the small peptide heptapeptide GHRP-1 and hexapeptide GHRP-6 as growth hormone secretagogue (GHS) has generated enormous interest in the search of non-peptidic small molecule growth hormone secretagogues (GHSs) because of its potential therapeutic applications such as age-related frailty.[1,2] A group of scientists from Merck reported the first non-peptidic growth hormone secretagogue L-692429. Subsequently, an orally active compound MK-0677 (**1**) was also reported from the same group.[3] Additional small molecule GHSs have been reported by pharmaceutical companies in the last several years.[3,4] Recently, we disclosed a potent, orally active GHS agonist, BMS-317180 (**2**), which was based on a tetrazole core and was advanced into pre-clinical development.[5] Although BMS-317180 showed excellent in vitro potency as well as excellent in vivo efficacy, it also showed a short half life and low exposure in pre-clinic pharmacokinetic studies. To address these potential PK liabilities associated with **2**, one possible modification was replacing the benzyloxy group of **2** with a more stable functionality.

The strategy of using a *gem*-difluoro benzyl group as a benzyloxy replacement to improve metabolic stability as well as pharmacokinetic properties of medically interesting molecules has been reported recently.[6] Those results inspired us to take a similar approach to address the potential pharmacokinetic issues which arose in preclinical studies of **2**. By employing a gem-difluoropropyl benzene as a replacement of the benzyloxy moiety of **2**, we hoped that this approach would improve the PK properties. To achieve this goal, a fluorine-containing amino acid, (*D*)-2-*tert*-butoxycarbonylamino-5,5-difluoro-5-phenyl-pentanoic acid **3**, was required as the starting material. However, to our best knowledge, there was no previous report of a synthesis of this amino acid. Herein we describe a concise synthesis of **3**, and analogs bearing a gem-difluoro benzyl group as a replacement of the left-hand O-benzyl side chain of **2** as well as related compounds. The initial biological and pharmacokinetic results are reported (Fig. 1).

As shown in Scheme 1, (*R*)-ethyl 5-oxopyrrolidine-2-carboxylate **4** was N-Boc protected, and subsequently treated with benzyl magnesium bromide to give the desired ketone **5** in 90% yield



1, MK-0677 **2, BMS-317180**

Figure 1. Small molecule growth hormone secretagogues: MK-0677 (**1**) and BMS-317180 (**2**).

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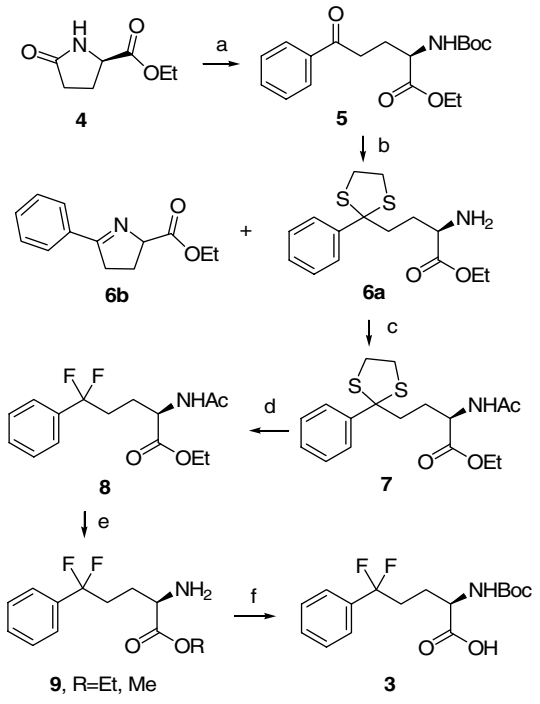
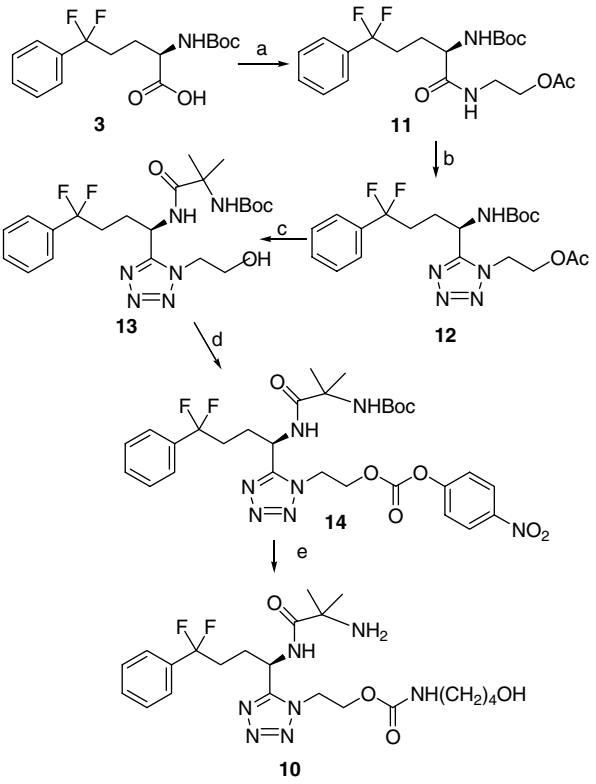


Scheme 1. Reagents and conditions: (a) i—Boc_2O, Et_3N, DMF (cat.), CH_2Cl_2, rt; ii—PhMgBr, −78 °C, THF, 85% (two steps); (b) 1.2-dithioethane, BF_3-Et_2O, 0 °C; (c) Ac_2O, Et_3N, CH_2Cl_2, 65% (two steps); (d) $NOBF_4$, HF/pyridine, CH_2Cl_2 50–65%; (e) sat. HCl/MeOH, 80 °C, 70%; (f) i—*t*-Boc_2O, Et_3N, DMAP (cat.), CH_2Cl_2; ii—LiOH/THF, 50% (two steps).



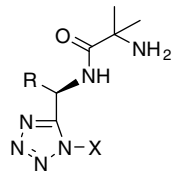
Scheme 2. Reagents and conditions: (a) i—NMM, iso-butyl-chloroformate, −40 °C; ii—2-amino-ethanol, −40 °C; iii—Ac_2O, pyridine, 96% (three steps); (b) $TMSN_3$, DEAD, Ph_3P, rt, 48 h, 70%; (c) i—HCl/dioxane, rt; ii—HOAt, EDAC, *N*-Boc-methylalanine, CH_2Cl_2, overnight; iii—1 N LiOH, 75% (two steps); (d) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2, 80%; (e) i—4-amino-1-butanol; ii—HCl/dioxane, 82% in two steps.

and ⩾99% ee.[7] Direct fluorination of the carbonyl functionality with various fluorinating reagents such as DAST or SF_6 failed to give the desired difluorinated adduct. We reasoned that the benzylic carbonyl group in **5** may not be active enough to react with these fluorination reagents. To facilitate fluorination, a dithiolation approach was adopted as reported by Olah.[8] However, dithiolation of **5** proved to be problematic under typical conditions due to the formation of the undesired cyclic compound **6b** as the major product. After trying several different conditions, we found that sequential addition of boron trifluoride-diethyl etherate at 0 °C to a mixture of **5** and excess 1,2-dithioethane resulted in a 70% yield of **6a**. The mixture of **6a** and **6b** was treated with Ac_2O/Et_3N and purified on a silica gel column to give the desired acetamide **7**. Having the amine protected as an acetamide was optimal since other protecting groups, such as Boc or Cbz, were found to be either incompatible with the next reaction or gave very low yields in the fluorination step. Treated compound **7** with $NOBF_4$ in HF/pyridine solution afforded desired gem-difluoro compound **8** in 50–65% yield.[8] Deprotection of acetamide was achieved by heating **8** in HCl/methanol in a sealed tube at 80.°C to give mixture of methyl/ethyl amino carboxylic ester **9** in ~70% yield. Re-protection of the primary amine with Boc, followed by hydrolysis of the mixed esters with LiOH in THF afforded the desired acid **3**.

As depicted in Scheme 2, synthesis of the gem-difluoro-bearing analog **10** was generated using previously described procedures.[5] Coupling of **3** with aminoethanol, followed by acylation of the crude amide gave the acetate **11** with quantitative yield. The formation of tetrazole **12** was achieved by sequential treatment of acetate **11** with one equivalent of triphenylphosphine, diethyl-azodi-carboxylate, and azidotrimethylsilane, followed by additional equivalents of each after 24 and 48 h, providing **12** in 80% isolated yield after silica gel chromatography. Boc-deprotection of **12**, amino acid coupling of the intermediate amine with *N*-Boc-aminoisobutyric acid (Boc-Aib) and lithium hydroxide hydrolysis of the acetate gave the corresponding alcohol **13** which was then treated with 4-nitrophenyl chloroformate to give carbonate **14** in 50% yield over four steps. Carbonate **14** was reacted with 4-amino-butanol to give the Boc-protected carbamate in high yield. Deprotection using 4 N HCl in dioxane gave the HCl salt of **10**, which was purified via preparative HPLC. Compound **16** was generated in a similar fashion.

Functional agonism of **2**, **10**, and **16** are depicted below for comparison. Interestingly, the potency of **10** (EC_{50} = 0.27 nM) was found to be significantly improved over that of parent compound **2** (EC_{50} = 1.92 nM) (Table 1). This finding indicated that the difluoro-methylene group in **10** enhanced binding affinity of the

Table 1
In vitro potency[a]



Compound[a]	R	X	EC_{50} (nM)
Ghrelin			1.42
2	$BnOCH_2$ –	$CH_2CH_2OC(O)N\ H(CH_2)_4OH$	1.92
10	$PhCF_2(CH_2)_2$ –	$CH_2CH_2OC(O)N\ H(CH_2)_4OH$	0.27
15	$BnOCH_2$ –	$(CH_2)_2CN$	30
16	$PhCF_2(CH_2)_2$ –	$(CH_2)_2CN$	0.90

[a] See Ref. 9 for detailed description.

Table 2
Pharmacokinetic results of **2** and **10** in rats

	IV (n = 3)		PO (n = 3)	
Compound	**2**	**10**	**2**	**10**
Dose (μmole/kg)	5	10	15	10
C_{max} (nM)			68	166
T_{max} (h)			0.7	1.6
AUC (nM*h)	401	901	110	239
T1/2 (h)	0.7	2.4		
MRT (h)	0.5	1.4		
Clearance (ml/min/kg)	213	183		
V_{ss} (L/kg)	6.4	16		
Bioavailability (%)			9.2	26

Values are means of three rats.

Table 3
In vivo activity of the compounds in the acute anesthetized rat model

Compounds	Increase GH[a] at 1.74 μmol/kg (% of vehicle)	Responders[b]
2	750(±112)	5/5
10	1934 (±130)	5/5

[a] Values are means of five experiments.
[b] A rat with more than 200% GH increase over vehicle control is considered as a responder.

molecule with the GHS receptor. One possibility is that the fluorines serve as hydrogen bonding acceptors with residues in the active sites. However, the detailed mechanism is still not clear since the fluorine hydrogen bond is a subject of controversy in different environments.[10] In another comparison, the gem-difluorinated analog **16** of an earlier program lead **15** was found to be substantially more potent as well (33-fold),[11] further corroborating the in vitro enhancement effected by this modification.

We then further evaluated the pharmacokinetic properties of **10** in fasted rats at an oral and IV dose of 10.μmol/kg. A comparison of PK parameters versus **2** is shown in Table 2. Upon oral dosing at 10 μmol/kg, compound **10** afforded a greater C_{max}, systemic exposure (AUC), and oral bioavailability as compared to compound **2**, which was orally dosed at 15 μmol/kg. The IV arm indicated that **10** also exhibited a longer half-life and slightly reduced clearance in the rat. These results suggest that the superior PK profile of this compound, versus **2**, may be attributed to the gem-difluoro benzyl functionality, which replaces the benzyloxy group found in **2**.

The efficacy of compounds **10** and **2** was further evaluated in an acute anesthetized rat model measuring endogenous GH release as the response. The compounds were administered intravenously at a dose of 1.74 μmol/kg. After 15 min, blood samples were collected and the plasma isolated and analyzed for rat growth hormone (GH). Data are expressed as the percentage of increase GH release compared with vehicle control animals (Table 3).

In agreement with the in vitro and PK findings, **10** showed improved in vivo activity in the acute anesthetized rat model, increasing rat GH by nearly 3-fold over that of the parent **2**.

In summary, a gem-difluoro benzyl group as a replacement of left-hand *O*-benzyl side chain of BMS-317180 **2** resulted in an analog **10**, which significantly improved the in vitro potency as well as in vivo efficacy in a rat model. Compound **10** also showed a greater C_{max}, systemic exposure (AUC), and oral bioavailability as compared to compound **2**, in the preliminary pharmacokinetic study in rats. Further application of the (D)-2-*tert*-butoxycarbonylamino-5,5-difluoro-5-phenyl-pentanoic acid **3** in the preparation other GHS compounds in the program will be reported in due course.

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