HETEROCYCLES, Vol. 64, 2004, pp. 437 - 446
Received, 7th October, 2004, Accepted, 30th November, 2004, Published online, 3rd December, 2004

DISCOVERY AND SYNTHESIS OF A POTENT, SELECTIVE AND ORALLY BIOAVAILABLE EP4 RECEPTOR AGONIST†

Robert N. Young,* Xavier Billot,[#] Yongxin Han, Deborah A. Slipetz, Nathalie Chauret, Michel Belley, Kathleen Metters, Marie-Claude Mathieu, Gillian M. Greig, Danielle Denis, and Mario Girard

Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe-Claire – Dorval, Québec, Canada H9R 4P8. E-mail: ryoung@merck.com
[#]Gemin X Biotechnologies Inc., P.O. Box 477, Place du Parc, Montreal, Québec, Canada, H2X 4A5

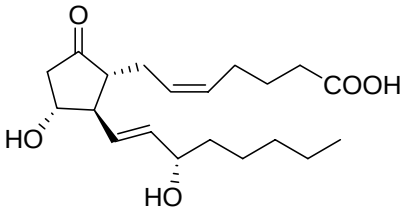
Abstract – An optimized analog of prostaglandin E_2 which incorporates a γ-lactam in place of the cyclopentanone ring and which incorporates metabolically stabilized side chains, has been identified and shown to exhibit potent and selective EP4 receptor agonism. The compound **(2)** (L-000902688) is also well absorbed on oral dosing and exhibits a long half-life making it an excellent tool for the study of the role of EP4 receptor in physiology and disease. An efficient synthesis of **2** from a chiral synthon is described.

INTRODUCTION

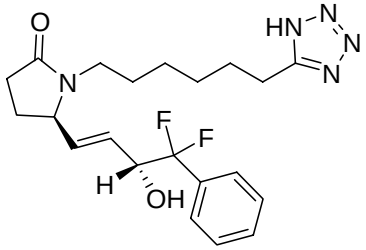
Prostaglandin E_2 (PGE_2) **(1)** is a potent lipid hormone which elicits a variety of tissue specific and sometimes conflicting biological activities in the body.[1] It is now known that PGE_2 interacts with four distinct receptors[2] and biological activities are presumably manifest depending on the predominant receptor subtype in a given tissue. Studies have shown that in vivo administration of PGE_2 can elicit bone growth in rats[3] and in humans[4] and this anabolic activity has been attributed to interaction of PGE_2 with the EP4 receptor subtype[5] which is found to be predominant in adult bone cells in rats.[5] Thus, it has been theorized that a selective EP4 agonist might provide a useful therapeutic to reverse the effects of osteoporosis and that such a therapeutic might lack the potential side effects which would be associated with interactions with the other EP receptors (EP1, EP2 and EP3).[6] We and others have recently reported the discovery of EP4 selective agonists[6,7] and reports have shown that such agonists can elicit bone

†Dedicated to Dr. Pierre Potier on the occasion of his 70th birthday

growth in animals.[8] Most of these agonists, however, exhibit short half-lives *in vivo* and/or are apparently poorly bioavailable when given by the oral route. This report details of the discovery and synthesis of (5*R*-[(1*E*)-4,4-difluoro-3*R*-hydroxy-4-phenylbut-1-en-1-yl]-1-[6-(1*H*-tetrazol-5-yl)hexyl]pyrrolidin-2-one (L-000902688) (**2**) which is not only a highly potent and selective agonist at the EP4 receptor but also embodies a degree of metabolic stability and solubility which allows it to be well absorbed and to exhibit a long half-life in rats. This compound should prove to be a useful tool to study the role of the EP4 receptor in physiology and disease.



PGE_2



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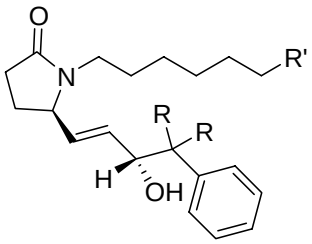


3a; R=H; R'=tetrazole
3b; R=CH_3; R'=COOH
3c; R,R= cypropyl; R'=COOH

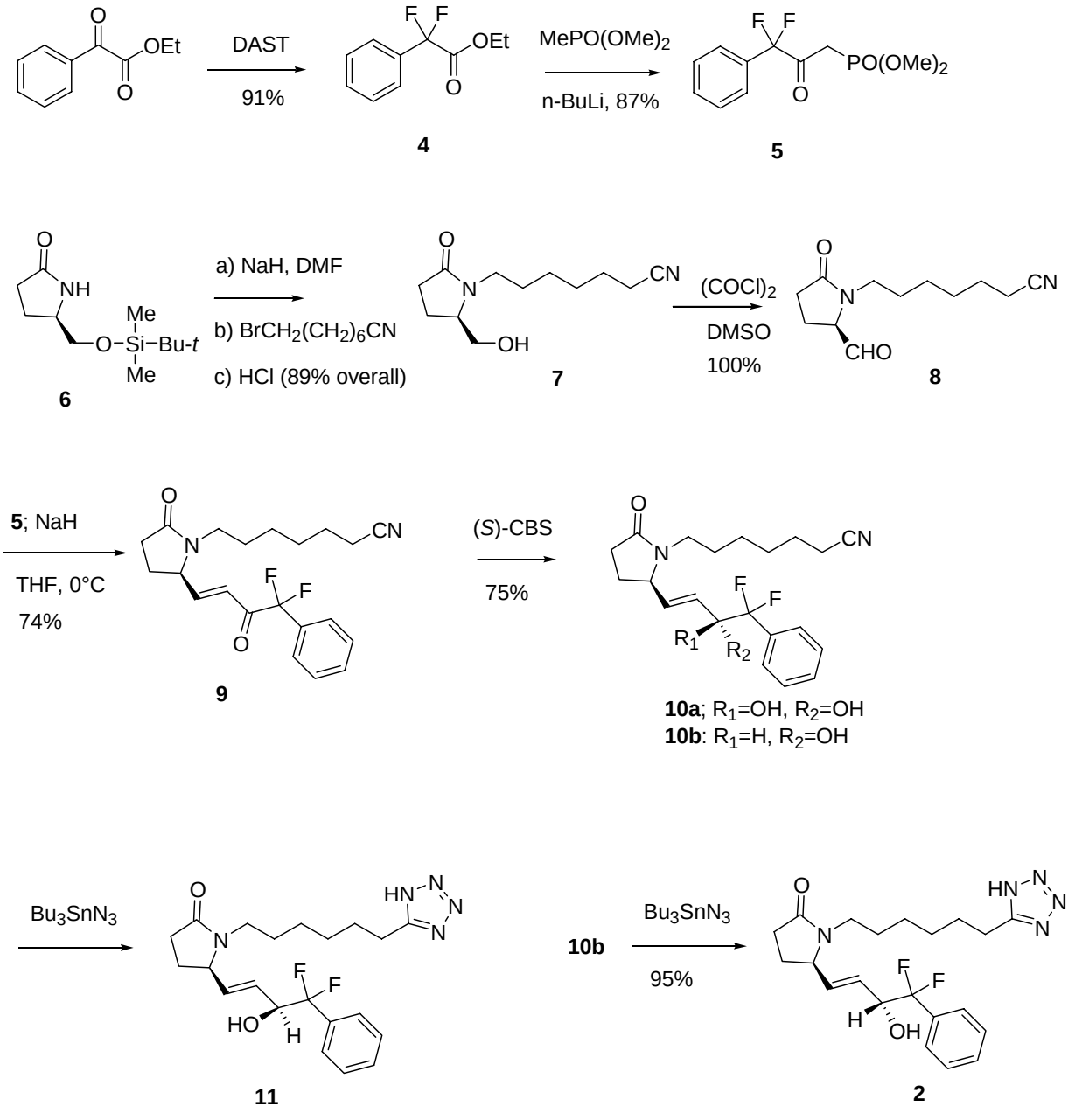
DISCUSSION

Previous studies identified compound (**3a**) as an EP4 selective agonist with a degree of metabolic stability and an *i.v.* terminal half-life of about two hours.[6] Unfortunately, when dosed orally to rats, the compound gave very low systemic blood levels. Considering that the compound contained a benzylic methylene group which was potentially susceptible to metabolic transformation, several alkyl substituted analogs (dimethyl (**3b**) and cyclopropyl (**3c**)) were previously prepared but were found to be significantly less potent than the parent compound **(3a)**.[6] In further studies, we decided to introduce a geminal difluoro group in this benzylic position, in part, based on the observation that the benzylic cyclopropyl analog **(3c)** was 5-fold more potent that the corresponding dimethyl analog (**3b)** (see Table 2). We surmised that the less sterically demanding difluoro analog could manifest further enhanced activity. We also noted reports in the literature suggesting that similar difluoro analogs were potent prostaglandin receptor agonists.[9]

Synthesis of compound (**2)** proceeded in a relatively straight-forward fashion starting from the previously prepared 7-(2*R*-formyl-5-oxopyrrolidin-1-yl)heptanenitrile (**8**). (Scheme). The synthesis of this aldehyde which was derived from the corresponding hydroxymethyl compound (**7**) using Swern oxidation,[10] could be carried out in a convenient fashion using anhydrous work-up conditions (see experimental). Under these conditions, the sensitive aldehyde (**8)** could be isolated directly in a non-hydrated form suitable for direct reaction in the next step. The aldehyde was reacted with the anion derived from dimethyl (3,3-difluoro-2-oxo-3-phenylpropyl)phosphonate (**5**) under standard Wittig-Horner conditions. (This

reagent was prepared from the corresponding keto-ester by reaction with DAST followed by addition of the anion of dimethyl methanephosphonate). The resulting ketone (**9**) was reduced with sodium

Scheme



borohydride to give a pair of diastereomeric alcohols (**10a,b**) in a ratio of *ca.* 2.5:1. These alcohols were separated using chiral column chromatography to provide the pure diastereomeric alcohols (**10a, 10b**) which were then reacted individually with tri-n-butylstannyl azide to provide the corresponding tetrazoles (**11, 2**). Unfortunately the minor isomer (**2)** was found to correspond to the compound with the greater

potency and which was subsequently shown to embody the natural *R*-stereochemistry at the 3-hydroxyl position (see Table 1). This was in contrast to experience in the synthesis of **3a,b** where the more potent isomer predominated under most reducing conditions. An extensive evaluation of a variety of selective reagents, many of which have been shown to provide the natural 15-*R*-hydroxyl stereochemistry in reduction of 15-keto analogs of prostaglandins (Table 1) was unsuccessful. Reduction with (*R)*-2-methyl-CBS-oxazaborolidine and catecholborane[11] at -45°C gave a 10:1 ratio again favoring the undesired diastereomer. However, the (*S)*-CBS reagent under the same conditions gave predominance of the desired isomer. The reaction gave the same result when a smaller amount (0.6 eq.) of (*S)*-CBS reagent was used. Separation of the diastereomers followed by conversion to the tetrazole yielded the desired compound (**2**). For ease of handling, the compound was converted to its calcium salt which was a non-hygroscopic solid suitable for *in vivo* studies.

Table 1: Reduction of difluoroketone 9

Conditions	Ratio **10a: 10b**
$NaBH_4$ (1.5 eq.), $CeC1_3$ (1 eq.), $EtOH/H_2O$, -20°C	2.5 : 1
L-Selectide (1.1 eq.), THF, -78°C	2 : 1
(S)-Alpine-Hydride (1.05 eq.), THF, -78°C	1 : 1 + other products
$Na(CN)BH_3$, MeOH/AcOH, 0°C	Mixture of 1,2- and 1,4-reduction
(R)-CBS (1 eq.), catecholborane (3 eq.), DCM, -40°C	10 : 1
(S)-CBS (1 eq.), catecholborane (3 eq.), DCM, -40°C	1 : 2
(S)-CBS(0.6 eq.), catecholborane (3.4 eq.), DCM, -40°C	1 : 1.8
(S)-CBS(0.1 eq.), catecholborane (3 eq.), DCM, -40°C	1 : 1 (incomplete reaction)

As noted in Table 2, **2** is very potent at the EP4 receptor with a Ki of 0.38 nM and also highly efficacious showing an EC50 of 0.6 nM and is thus 3 to 5-fold more potent at the EP4 receptor than both the previously optimized agonist compound (**3a)** and PGE_2 itself.

Evaluated in *in vitro* incubations with rat and human hepatocytes, compound (**2**) showed a high degree of metabolic stability and provided only small amounts of metabolites. The major metabolites had molecular weights indicative of them being glucuronide conjugates presumably either at the tetrazole or the hydroxyl function. Small amounts of oxidative metabolites (M + 16) were also observed but not

characterized. Compound (**2)** was found to have good oral bioavailability in the rat (15%) with a long half-life (*ca.*12 h). When dosed orally to rats for four days at 1 mg/kg, blood levels peaked at approximately 2 h after dosing at 20-25 nM and residual blood levels at time 0 on the fourth day (24 hr after dosing on day 3) were 5 nM. Thus this dose can provide greater than 10 fold multiples of the EC_{50} throughout the dosing period. The rats, however, exhibited signs of abdominal discomfort and weight loss suggesting that at least some of the gastrointestinal side effects associated with PGE2 may be mediated through the EP4 receptor. Lower doses (0.1 and 0.3 mg/kg) were better tolerated and thus this compound could serve as a useful tool to explore the *in vivo* effects of EP4 agonism in physiology. Studies carried out to examine the effects of **2** on bone will be reported elsewhere.

Table 2: Agonist potency and radioligand binding affinity and selectivity.

Compound	**Agonist EC50 (nM)**	**Radioligand Binding Ki (nM)**							
	EP4	EP4	EP1	EP2	EP3-III	DP	FP	IP	TP
2	0.6 ± 0.1	0.38 ± 0.02	>12000	3200	2800	>4800	>8000	>6000	>6000
11	1190(n=1)	690(n=1)							
3a	2.5 ± 1.0	1.2 ± 0.2	>13,000	>13,000	>13,000	>13,000	>13,000	>13,000	>13,000
3b	nt	62(n=1)	>13,000	>13,000	10560	>13,000	>13,000	>13,000	>13,000
3c	nt	12(n=1)	>13,000	>13,000	3340	>13,000	>13,000	>13,000	>13,000
PGE_2	3.0±0.4	0.8±0.07	9.1±1.5	4.9± 0.5	0.33±0.3	307±106	119±12	>100000	>20,000

EXPERIMENTAL

Biological Assays

Receptor binding assays were performed using cell membranes from HEK 293ebna cells recombinantly expressing the corresponding human prostanoid cDNA's.[12] EP4 agonist potency and efficacy were evaluated utilizing a stable clone of pSV40-EP4 transfected into HEK293 cells that expresses approximately 50 fmol/mg EP4 receptor. Whole cell cAMP assays were performed essentially as described in Slipetz *et al*.[13] with the following modifications. Assays were performed with cells in suspension in a total of 0.2 mL HBSS containing 2 mM IBMX (phosphodiesterase type IV inhibitor).

IBMX and PGE_2 or the test compound were added to the incubation mixture in DMSO to a final vehicle concentration of 1.8% (v/v) (kept constant in all samples). The reaction was initiated by the addition of 1 x 10^5 cells per incubation, samples were incubated at 37°C for 10 min, and the reaction was terminated by immersing the samples in boiling water for 3 min. Measurement of cAMP was performed by a [^{125}I]cAMP scintillation proximity assay. For *in vivo* and *in vitro* metabolism methods, see methods described by Nicoll-Griffith *et al.*[14]

General Chemistry

NMR spectra were recorded as solutions in deuterochloroform or acetone-d_6, unless otherwise stated, and at field strength stated and using the solvent as internal standard. Preparative scale HPLC for enantiomer separation was carried out using a ChiralPak AD™ (silica, coated with amylose tris(3,5-dimethylphenyl carbamate)) HPLC columns from Chiral Technologies.

The chirality and relative stereochemistry of compound (**10b**) were determined by X-Ray analysis.

Ethyl difluoro(phenyl)acetate (4)- To a solution of ethyl benzoylformate (82 g, 460 mmol) in toluene (100 mL) was added diethylaminosulfur trifluoride (DAST, 127 mL, 961 mmol) portionwise at rt (1/8 of total amount each time, at 15-30 min intervals between each addition for the exothermic reaction to subside). After addition of the last portion of DAST, the mixture was stirred for another 4 h at rt, after which time it was poured into ice containing conc. NH_4OH (500 mL). The mixture was then neutralized with sat NH_4Cl (1 L) and conc. HCl and the product was extracted into EtOAc. The organic layer was washed with 2N HCl, dried over Na_2SO_4 and evaporated. Pure acetate **(4)** (84.17 g, 91%) was obtained after distillation (110-115°C, 25 mmHg). ^{1}H NMR (500 MHz, acetone-d_6) δ 7.62 (d, J = 9 Hz, 2H), 7.55 (m, 3H), 4.35 (q, J = 6 Hz, 2H), 1.27 (t, J = 6 Hz, 3H).

Dimethyl (3,3-difluoro-2-oxo-3-phenylpropyl)phosphonate (5) - To a solution of dimethyl methanephosphonate (50 mL, 460 mmol) in THF (700 mL) was added dropwise n-BuLi (2.5 M in hexanes, 185 mL) at -78°C and this solution was stirred for another 20 min at the same temperature. Then, a solution of acetate **(4)** (84.1 g, 420 mmol) in THF (200 mL) was added at -78°C and the mixture was stirred for another 4 h at the same temperature. The reaction was quenched by addition of a solution of acetic acid (60 mL) in THF (100 mL) at -78°C, followed by a half-saturated solution of NH_4Cl at rt. The product was extracted into i-PrOAc, dried over Na_2SO_4 and concentrated to dryness. Traces of solvents were removed by co-evaporation with toluene and drying under high vacuum to yield the crude product (**5)** (101.91 g, 87%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65-7.40 (m, 5H), 3.77 (s, 3H), 3.74 (s, 3H), 3.35 (d, J = 22 Hz, 2H); MS 279.1 (M+1).

7-[(2*R*)-2-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-5-oxopyrrolidin-1-yl]heptanenitrile - To a mixture of NaH (60% in oil, 34.7 g, 870 mmol) in DMF (600 mL) was added a solution of (5*R*)-5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyrrolidin-2-one **(6)**[15] (142.2 g, 620 mmol) in DMF (600 mL) at rt Then, sodium iodide (10 g, 667 mmol) and 7-bromoheptanenitrile (158 g, 828 mmol) were added and the mixture was stirred between 30 and 40°C for 30 min (with cooling to control the exothermic reaction) and then at 50°C for 2 h and at rt overnight. The mixture was poured into 3 L of ice-cold half-saturated NH_4Cl and the products were extracted in EtOAc. (2x1.5 L). The combined organic solutions were washed with water (2x1.6 L) and brine (1 L), dried over Na_2SO_4, filtered, and concentrated. Purification of a small sample by flash chromatography on silica gel eluting with a 20% acetone:toluene provided pure 7-[(2*R*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-5-oxopyrrolidin-1-yl]heptanenitrile as an oil. ^{1}H NMR (500 MHz, acetone-d_6) δ 3.85 (dd, J = 2, 10 Hz, 1H), 3.75 (m, 1H), 3.69 (dd, J = 3, 10 Hz, 1H), 3.48 (m, 1H), 3.04 (m, 1H), 2.48 (t, J = 8 Hz, 2H), 2.30 (m, 1H), 2.13 (m, 2H), 1.86 (m, 1H), 1.70-1.42 (m, 6H), 1.34 (m, 2H), 0.92 (s, 9H), 0.10 (2s, 6H); MS 339.3 (M+1).

7-[(2*R*)-2-Hydroxymethyl-5-oxopyrrolidin-1-yl]heptanenitrile (7) – To the crude 7-[(2*R*)-2-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-5-oxopyrrolidin-1-yl]heptanenitrile dissolved in THF (1.2 L) was added 1N HCl (300 mL). The mixture was stirred at rt for 3 days, then the solvents were evaporated to dryness and the remaining traces of solvents co-evaporated twice with toluene. Purification by flash chromatography on silica gel (2.8 kg) eluting with a solvent gradient (acetone:toluene 40:60 to acetone:ethanol:toluene 65:10:25) provided **7** (123.8 g, 89%, 2 steps) as an oil. ^{1}H NMR (500 MHz, acetone-d_6) δ 3.78 (m, 2H), 3.60 (m, 2H), 3.08 (m, 1H), 2.47 (t, J = 7 Hz, 2H), 2.38 (m, 1H), 2.25 (m, 1H), 2.12 (m, 1H), 1.93 (m, 1H), 1.65 (m, 3H), 1.50 (m, 3H), 1.37 (m, 2H); MS 225.1 (M+1).

7-[(2*R*)-2-Formyl-5-oxopyrrolidin-1-yl]heptanenitrile (8) - Oxalyl chloride (34 mL, 388 mmol) was added portionwise to a solution of DMSO (30 mL, 423 mmol) in CH_2Cl_2 (500 mL) at -72°C and the mixture was stirred at this temperature for 20 min. Then, a solution of the alcohol **(7)** (73.16 g, 326 mmol) in CH_2Cl_2 (200 mL) was added slowly and the mixture was stirred at -72°C for 30 min. Triethylamine (135 mL, 960 mmol) was then added slowly and the mixture was stirred again at -72°C for 45 min after which it was warmed to -15°C. Ether (800 mL) was added and the solid $Et_3N{\cdot}HCl$ was removed by filtration through celite and was washed with cold ether:CH_2Cl_2 1:1 (800 mL). The solvents were evaporated and the remaining oil was redissolved in THF:ether 1:1 (600 mL) and filtered again to remove more $Et_3N{\cdot}HCl$. Evaporation of the solvents afforded the crude aldehyde (**8**) (72.5 g, 326 mmol) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 4.15 (m, 1H), 3.65 (m, 1H), 3.10 (m, 1H), 2.45 (m, 2H), 2.35 (m, 3H), 2.10 (m, 1H), 1.68 (m, 2H), 1.52 (m, 4H), 1.35 (m, 2H).

7-{(2*R*)-2-[(1*E*)-4,4-Difluoro-3-oxo-4-phenylbut-1-en-1-yl]-5-oxopyrrolidin-1-yl}heptanenitrile (9) -

To a solution of the phosphonate **(5)** (90.7 g, 326 mmol) in THF (500 mL) was added NaH 60% in oil (13 g, 325 mmol) at 0°C, and the reaction mixture was stirred at rt for 1.5 h. Then, at -10°C, a solution of crude aldehyde **(8)** (72.5 g, 326 mmol) in THF (300 mL) was added and the resulting mixture was stirred at rt overnight. A half-saturated solution of NH_4Cl (800 mL) was added and the product was extracted with i-PrOAc, dried over Na_2SO_4, concentrated and purified by filtration through silica gel (1.8 kg) using a gradient of EtOAc:hexanes (65 to 100%) as eluent to yield **9** (90.33 g, 74%) as an oil. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.60 (m, 5H), 7.05 (dd, J=10, 14 Hz, 1H), 6.82 (d, J=14 Hz, 1H), 4.44 (m, 1H), 3.45 (m, 1H), 2.82 (m, 1H), 2.47 (t, J=7 Hz, 2H), 2.40-2.20 (m, 3H), 1.84 (m, 1H), 1.61 (m, 2H), 1.43 (m, 4H), 1.26 (m, 2H).

7-{(2*R*)-2-[(1*E*,3*R*)-4,4-Difluoro-3-hydroxy-4-phenylbut-1-en-1-yl]-5-oxopyrrolidin-1-yl}heptanenitrile (10b) - To a solution of the ketone **(9)** (27.74 g, 74.1 mmol) and (*S*)-2-methyl-CBS-oxazaborolidine (47 mL of a 1M solution in toluene) in CH_2Cl_2 (340 mL) maintained at -40°C was added slowly a 1M solution of catecholborane in THF (250 mL). The mixture was then warmed up slowly to -20°C, at which point the reaction was found to be complete by TLC. The reaction was quenched with 2N HCl and the mixture was stirred at rt overnight. Methanol (75 mL) was then added and the resulting mixture was filtered through celite. The organic layer was separated and the aqueous layer was reextracted with i-PrOAc. The combined organic phases were dried over Na_2SO_4, concentrated and purified by filtration through silica gel using a gradient of EtOAc:hexanes (60 to 100%) and ethanol:EtOAc 15% as eluents to yield an oil (21 g, 75%) consisting of a 1.8:1 mixture of **10b** and its 3*S* isomer (**10a)**. The two isomers were separated by chiral HPLC on a ChiralPak AD column (20 µm, 5x50 cm, eluent 25% i-PrOH/hexane, flow rate 80 mL/min, detection at 264 nm; on an analytical column eluting with 20% i-PrOH/hexane at 1 mL/min, the retention time of the 3*S* isomer (**10a)** and compound (**10b)** are respectively 10.2 and 16.5 min). The fractions containing the more polar isomer (**10b)** were concentrated and the crystallized solid triturated in ether. mp 89-90°C, ^{1}H NMR (500 MHz, acetone-d_6) δ 7.52 (m, 5H), 5.72 (m, 2H), 5.04 (br s, 1H, OH), 4.67 (m, 1H), 4.14 (m, 1H), 3.42 (m, 1H), 2.72 (m, 1H), 2.47 (t, J = 7 Hz, 2H), 2.20 (m, 3H), 1.65 (m, 3H), 1.45 (m, 4H), 1.28 (m, 2H).

(5*R*)-5-[(1*E*,3*R*)-4,4-Difluoro-3-hydroxy-4-phenylbut-1-en-1-yl]-1-[6-(1*H*-tetrazol-5-yl)hexyl]pyrrolidin-2-one (2) – To the nitrile **(10b)** (31.82 g, 84.5 mmol) was added azidotributyltin (45.3 g, 136 mmol) and the mixture was heated to 120°C for 6 h. The reaction mixture was then dissolved in boiling acetone (150 mL), then diluted with toluene (150 mL) and purified by flash chromatography on silica gel (1 kg) with a gradient of acetone:toluene:AcOH 20:80:0 to 40:60:2 as eluent to yield **2** (33.67 g, 95%) as an oil. ^{1}H NMR (500 MHz, acetone-d_6) δ 7.57-7.47 (m, 5H), 5.72 (m, 2H), 4.67 (m, 1H), 4.17 (m, H), 3.42 (m, 1H), 2.97 (t, J = 7 Hz, 2H), 2.73 (m, 1H), 2.30-2.17 (m, 3H), 1.80 (m, 2H), 1.66 (m, 1H), 1.42 (m, 4H) 1.30 (m, 2H); MS 420.3 (M+1).

Calcium salt of (5*R*)-5-[(1*E*,3*R*)-4,4-difluoro-3-hydroxy-4-phenylbut-1-en-1-yl]-1-[6-(1*H*-tetrazol-5-yl)hexyl]pyrrolidin-2-one - The tetrazole (**2)** (37.64 g, 89.7 mmol) was dissolved in a small amount of hot methanol and diluted with deionized water. Calcium hydroxide (3.996 g, 54 mmol) was then added and the mixture was stirred at rt for 30 min. The excess amount of calcium hydroxide was removed by filtration and the methanol was evaporated. The aqueous solution was washed twice with ether and the product was freeze-dried. The gummy solid was finally triturated into acetone:hexane 3:1 (270 mL), filtered under nitrogen and dried under high vacuum to yield a white solid (35.48 g, 90%), mp 140°C (decomp). ^{1}H NMR (500 MHz, methanol-d_4) δ 7.52-7.42 (m, 5H), 5.80 (dd, J = 7, 15 Hz, 1H), 5.64 (dd, J = 10, 15 Hz, 1H), 4.55 (m, 1H), 4.15 (m, H), 3.38 (m, 1H), 2.85 (t, J = 7 Hz, 2H), 2.72 (m, 1H), 2.36 (m, 2H), 2.24 (m, 1H), 1.76 (m, 2H), 1.68 (m, 1H), 1.50-1.32 (m, 4H) 1.28 (m, 2H).

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