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Regioselective Epoxide Ring Opening for the Stereospecific Scale-Up Synthesis of BMS-960, a Potent and Selective Isoxazole-Containing $S1P_1$ Receptor Agonist

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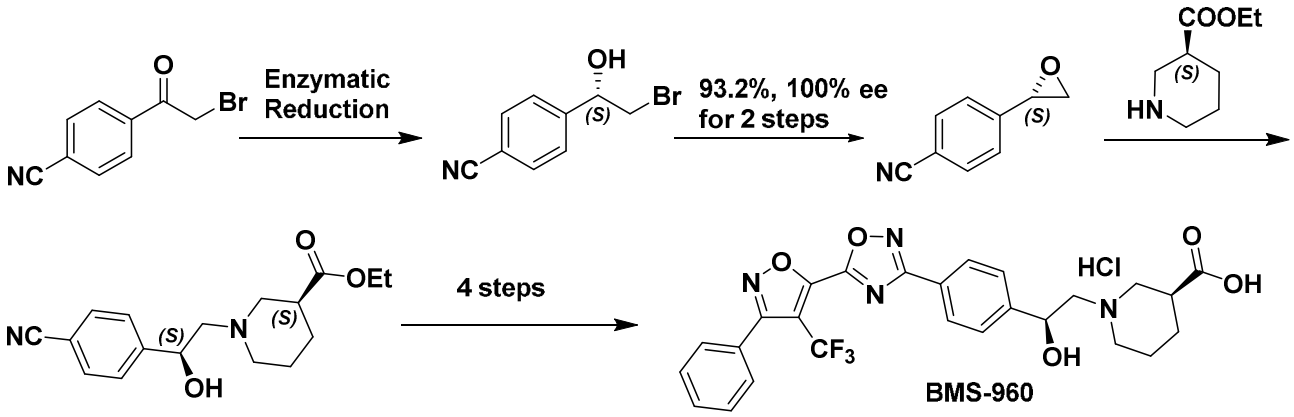


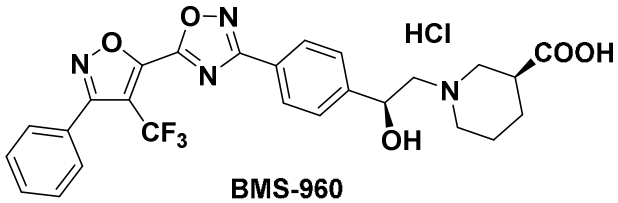
ABSTRACT: This article presents a stereospecific scale-up synthesis of (*S*)-1-((*S*)-2-hydroxy-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylic acid (BMS-960), a potent and selective isoxazole-containing $S1P_1$ receptor agonist. The process highlights an enzymatic reduction of α-bromo ketone toward the preparation of (*S*)-bromo alcohol, a key precursor of (*S*)-4-(oxiran-2-yl)benzonitrile. A regio-selective and stereo-specific epoxide ring opening reaction was also optimized along with improvements to 1,2,4-oxadiazole formation, hydrolysis and crystallization. The improved process was utilized to synthesize batches of BMS-960 for Ames testing and other toxicological studies.

KEYWORDS: Enzymatic reduction, Epoxide ring opening, 1,2,4-oxadiazole, Stereospecific synthesis, $S1P_1$ receptor agonist, Process optimization.

INTRODUCTION

Sphingosine-1-phosphate (S1P) is the endogenous ligand for the sphingosine-1-phophate receptors ($S1P_{1-5}$) and triggers a number of cellular responses through their stimulation. S1P and its interaction with the S1P receptors play a significant role in a variety of biological processes including vascular stabilization, heart development, lymphocyte homing, and cancer angiogenesis. Agonism of $S1P_1$, especially, has been shown to play an important role in lymphocyte trafficking from the thymus and secondary lymphoid organs, inducing immunosuppression, which has been established as a novel mechanism of treatment for immune diseases and vascular diseases.[1-7]

During the course of our $S1P_1$ receptor agonist program, BMS-960 (Figure 1) was identified as a lead backup candidate for further biological and toxicological studies.[8] Herein, we report an efficient stereoselective scale up synthesis of BMS-960 to supply the sufficient amount of API for such studies.



BMS-960

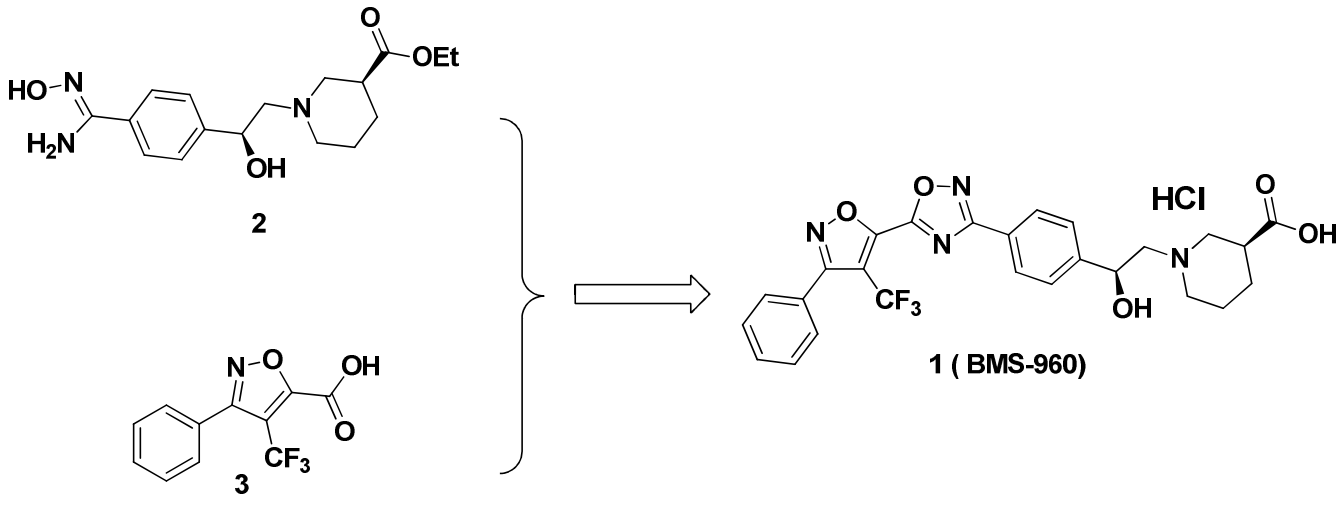
Figure 1. (*S*)-1-((*S*)-2-hydroxy-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylic acid, HCl (BMS-960)

RESULTS AND DISCUSSIONS

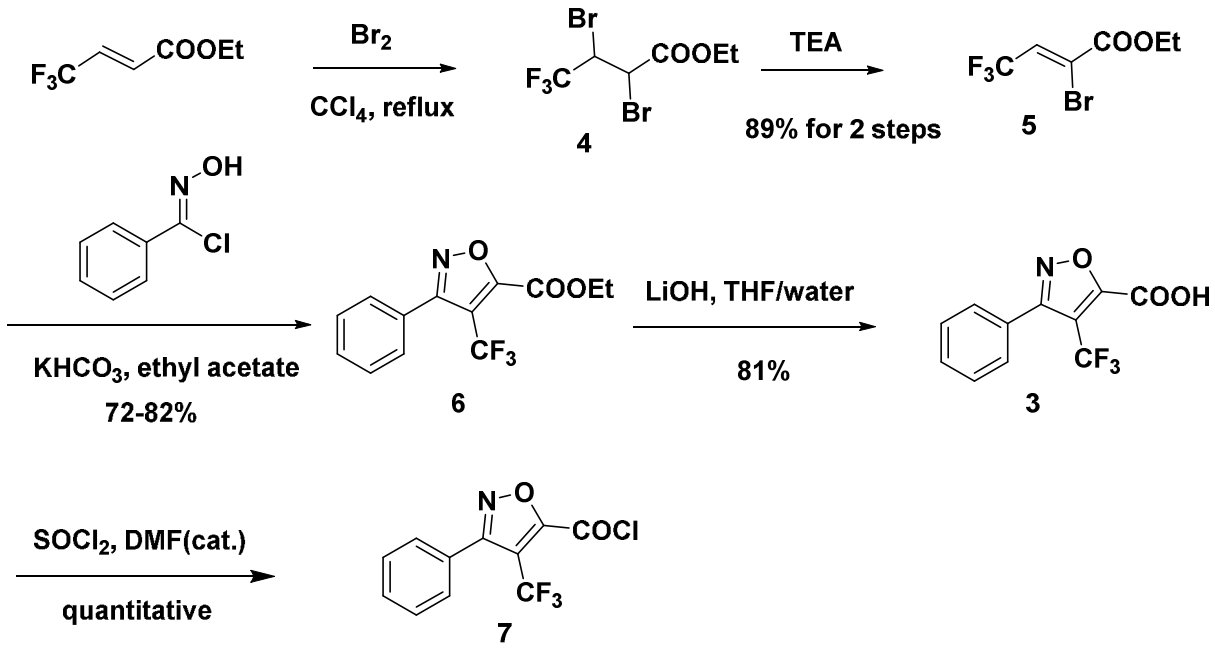
Original Synthesis of (*S*)-1-((*S*)-2-hydroxy-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylic acid 1 (BMS-960). To prepare (*S*)-1-((*S*)-2-hydroxy-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl) piperidine-3-carboxylic acid (BMS-960), a convergent synthesis (Scheme 1)[8] was developed, which required two advanced intermediates, ((*S*)-ethyl 1-((*S*)-2-hydroxy-2-(4-((Z)-N'-hydroxycarbamimidoyl)phenyl)ethyl)piperidine-3-carboxylate **2** and 3-phenyl-4-

(trifluoromethyl)isoxazole-5-carboxylic acid **3**. During the course of our synthesis of the first generation candidate, BMS-520,[10a] we developed a robust, scalable and highly regioselective [3+2]-cycloaddition and a chemo-selective hydrolysis, which enabled us to efficiently prepare the carboxylic acid **3** and the corresponding acyl chloride **7** in large scale with high purity (Scheme 2).

Scheme 1: Convergent Synthesis of BMS-960



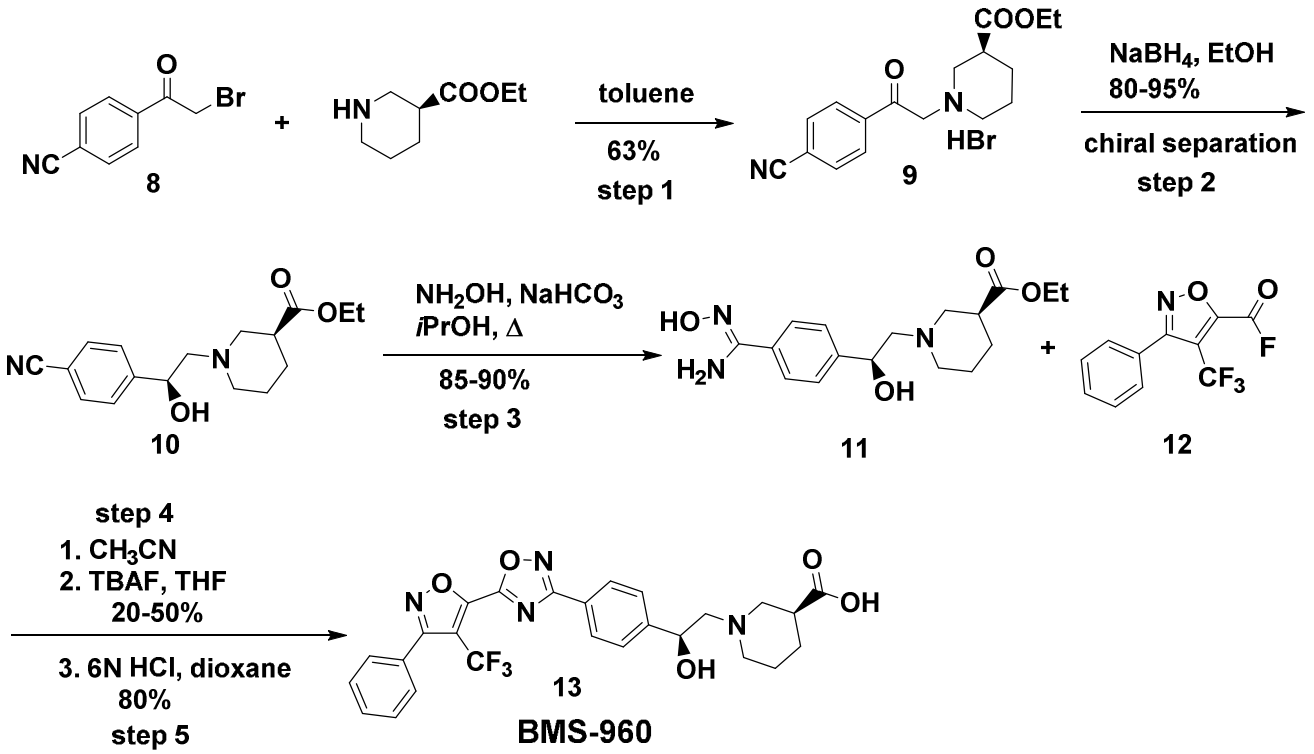
Scheme 2: 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid **3**.[10]



Originally, a small amount of BMS-960 was prepared for preliminary biological evaluation through a non-selective reduction and chiral separation (Scheme 3, step 2). Although the original route

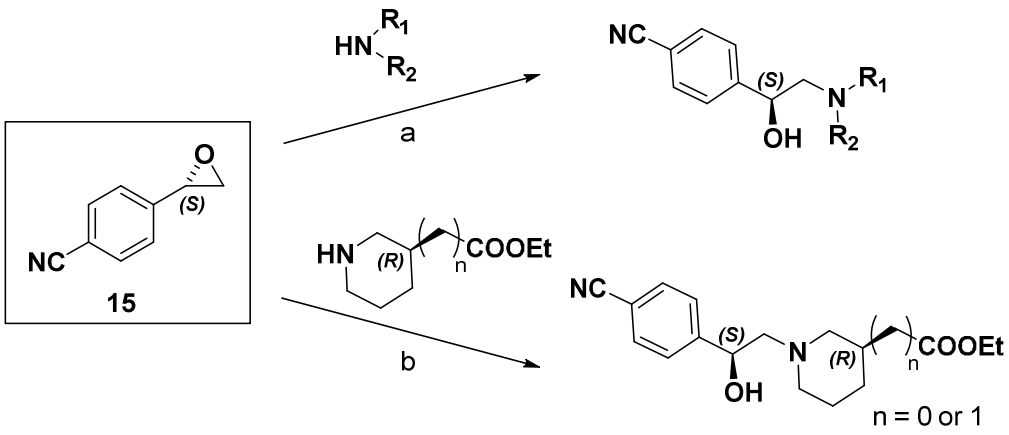
was amenable for a small scale preparation of analogues, there were a few issues to be addressed before it could be efficiently scaled up to meet requirements of toxicological studies in terms of API quality and quantity: a) step 1 N-alkylation yield was only moderate. b) step 3 used isopropyl alcohol which generates the ester exchange byproduct. c) step 4 oxadiazole formation yield was low, probably due to the presence of an unprotected OH group. d) acyl fluoride was prepared from an unfavorable reagent, cyanuric fluoride, which is very caustic and could even corrode the glassware during scale-up. Additionally, a limitation of the original route was that it could not offer a common intermediate for the synthesis of other substituted piperidine candidates or diversification of analogues.

Scheme 3: Original Synthesis of BMS-960.[8]



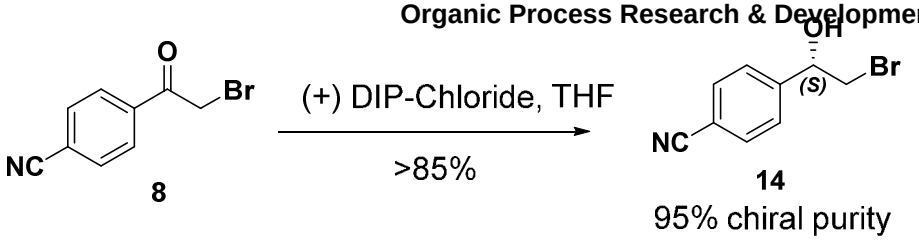
To address the above issues, an alternative synthesis was proposed to efficiently scale up BMS-960 (Scheme 7). Strategically, (*S*)-4-(oxiran-2-yl)benzonitrile **(15)** served as a key common intermediate, which would allow for later stage modifications. This common intermediate was likely to expedite both the medicinal chemistry parallel synthesis (Scheme 4, a) and later scale-up campaigns (Scheme 4, b) within the same β-amino alcohol chemotype.

Scheme 4: the common intermediate approach.



Our initial literature searches indicated that epoxides could be quickly prepared in a single step from the corresponding aldehydes under a condition of trimethylsulfonium iodide/KOH.[9] However, the yield was only 37% for our substrate, 4-formylbenzonitrile. Alternatively, we proposed a two-step method for epoxide formation. Starting with the commercial prochiral α-bromoketone **8**, we initially piloted a racemic reduction with $NaBH_4$ to quickly access the racemic secondary alcohol **14** for later stage chemistry exploration**.** We noticed that the secondary alcohol **14** was unstable and was directly converted to the racemic epoxide **15** under basic conditions with 90% yield for the two steps. However, chiral separation was necessary to obtain the chirally pure desired (*S*)-epoxide **15,** which was not favorable for a large scale. We therefore shifted our focus to an asymmetric reduction [11-13] to prepare optically pure alcohol **14.** Considering the scale, cost and availability, we selected *B*-chlorodiisopinocampheylborane (Aldrich: DIP-Chloride™) as a chiral reducing agent [14,15] to convert prochiral ketone **8** to the chiral alcohol **14** (Scheme 5). The preliminary results were very encouraging. (+) DIP-Chloride™ gave the desired enantiomer **14** with 95% chiral purity and >85% chemical conversion under mild conditions (0°C to RT). Despite these encouraging results, chiral separation was still needed to remove the residual undesired enantiomer and so we decided to pursue an enzymatic reduction with the goal of achieving the same reduction with even higher enantiomeric excess.

Scheme 5: Asymmetic chemical reduction of bromoketone **8**



To this end, the enzymatic reduction of α-bromoketone **8** to α-bromohydrin **14** followed by facile formation of epoxide **15** now became our focus.[16,17] At the beginning, we explored the enzyme reduction of (*S*)-ethyl 1-(2-(4-cyanophenyl)-2-oxoethyl)piperidine-3-carboxylate hydrobromide **9** to the chiral alcohol **10** in the original synthesis of BMS-960 (Scheme 3). Initial screening of a number of both NADH- and NADPH-dependent ketoreductases proved to be very encouraging. Ketoreductases with (*S*)- and (*R*)-diastereoselectivity were identified producing either the desired chiral (*S*)-alcohol **10** or the undesired chiral (*R*)-alcohol respectively. Eight ketoreductases (KRED-106, KRED-111, KRED-114, KRED-115, KRED-127, KRED-145, ES-KRED-109 and ES-KRED-136) showed high conversion and diastereomeric excess (de) for **10**. In order to accommodate the needs of the common intermediate approach (Scheme 6), we carried out enzyme screening for the reduction of α-bromoketone **8** with appropriate cofactor. The reaction medium for NADPH dependent ketoreductases contained 250 mM potassium phosphate (pH 7.0), 0.5 mM dithiothreitol, 2 mM magnesium sulfate, 1.1 mM $NADP^+$, 80 mM D-glucose, 10 U/mL glucose dehydrogenase. Whereas, screening of NADH-dependent enzymes was performed in a medium which was composed of the same above components except that 1.3 mM NAD^+ rather than 1.1 mM $NADP^+$ was used. In general, screening experiments were set up with 0.5 mL of reaction medium, 1 mg enzyme and a stock solution of α-bromoketone **8** 5.0 mg in 10 µL DMSO. The reaction mixtures were stirred at 30°C and 300 RPM for 24 hours. The crude reaction mixtures were filtered and analyzed by HPLC to determine the chemical conversion and ee. Among the approximate 250 ketoreductase enzymes screened, seventeen ketoreductase enzymes showed >99% conversion with >99% ee for the desired (*S*)-alcohol **14** (Table 1). On the other hand, twenty one ketoreductase enzymes showed >95% conversion with >95% ee for the undesired (*R*)-alcohol **18** (Table 2). Many other ketoreductases showed lower conversions of the α-bromoketone **8** producing either

alcohol **14** or **18** with varying degree of selectivities. Ultimately, ketoreductase enzyme KRED-NADH-110 was selected for the reduction of α-bromoketone **8** to (*S*)-alcohol **14** based on its activity, selectivity, and substrate to enzyme ratio. Though both NADH and NADPH cofactors have to be regenerated for cost reduction, KRED-NADH-110 also has the additional advantage of requiring the lower cost NADH co-factor.

Table 1: Screening of Ketoreductases - Enzymes producing chiral (*S*)-alcohol **14** preferentially

Ketoreductase	Supplier	Cofactor	Conversion (%)	ee (%) of 14
CRED-A601	ALMAC	NADPH	100	100
CRED-A311	ALMAC	NADPH	100	100
CRED-A291	ALMAC	NADPH	100	99.7
CRED-A401	ALMAC	NADPH	100	99.7
CRED-A161	ALMAC	NADH	100	100
ES-KRED-119	EnzySource	NADPH	100	99.4
ES-KRED-135	EnzySource	NADPH	100	99.2
ES-KRED-126	EnzySource	NADH	100	99.2
KRED-136	Codexis	NADPH	100	100
KRED-149	Codexis	NADPH	100	100
KRED-150	Codexis	NADPH	100	100
KRED-155	Codexis	NADPH	100	100
KRED-162	Codexis	NADPH	100	100
KRED-177	Codexis	NADPH	100	100
KRED-NADH-110	Codexis	NADH	100	100
KRED-NADH-111	Codexis	NADH	100	99.1
KRED-NADH-124	Codexis	NADH	100	99.2

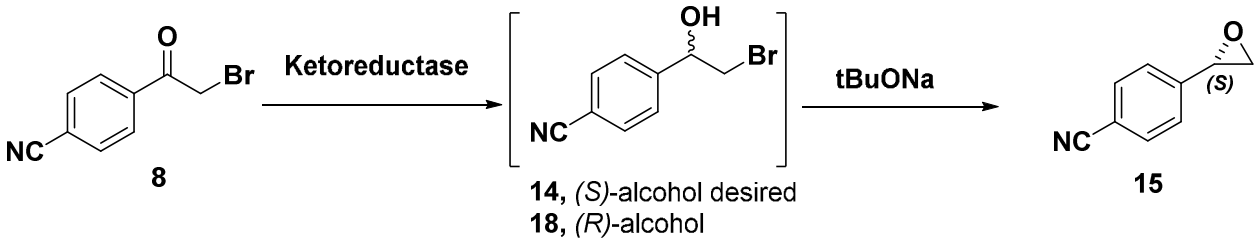
Table 2: Screening of Ketoreductases - Enzymes producing chiral (*R*)-alcohol **18** preferentially

Ketoreductase	Supplier	Cofactor	Conversion (%)	ee (%) of 18
CRED-A281	ALMAC	NADPH	100	100
CRED-A181	ALMAC	NADPH	100	98
CRED-A371	ALMAC	NADPH	99	100
CRED-A131	ALMAC	NADH	98	100
ES-KRED-101	EnzySource	NADPH	100	96
ES-KRED-127	EnzySource	NADPH	96	97
KRED-104	Codexis	NADPH	99	100
KRED-130	Codexis	NADPH	100	100
KRED-131	Codexis	NADPH	99	98
KRED-132	Codexis	NADPH	100	100

KRED-142	Codexis	NADPH	100	100
KRED-148	Codexis	NADPH	100	100
KRED-161	Codexis	NADPH	100	100
KRED-NADH-102	Codexis	NADH	98	100
KRED-NADH-101	Codexis	NADH	96	96
KRED-NADH-105	Codexis	NADH	99	96
KRED-NADH-108	Codexis	NADH	100	98
KRED-NADH-112	Codexis	NADH	100	100
KRED-NADH-117	Codexis	NADH	98	100
KRED-NADH-123	Codexis	NADH	100	100
KRED-NADH-126	Codexis	NADH	100	100

Under basic conditions, the chiral alcohol **14** was readily transformed into (*S*)-epoxide 1**5** (Scheme 6). The conditions for the reduction of α-bromoketone **8** to the chiral alcohol and the conversion to the (*S*)-epoxide 1**5** were subsequently optimized in terms of chemical conversion, ee, substrate to enzyme ratio, temperature, and reaction time. A very low loading of ketoreductase KRED-NADH-110 was sufficient to effect the complete reduction of 100g of α-bromoketone **8** in 5 hours at 40^{o}C at a substrate concentration of 40g/L with a substrate to enzyme ratio of 200:1 and substrate to NAD cofactor ratio of 25:1. The cofactor was regenerated by a small amount (8 kU) of the glucose dehydrogenase enzyme and glucose (96g). The chiral alcohol **14** was extracted in MTBE, then readily converted to (*S*)-epoxide **15** by sodium tert-butoxide. After work up, the pure chiral (*S*)-epoxide **15** was isolated as a stable solid in 93% yield, 99.9% AP, 100% ee.

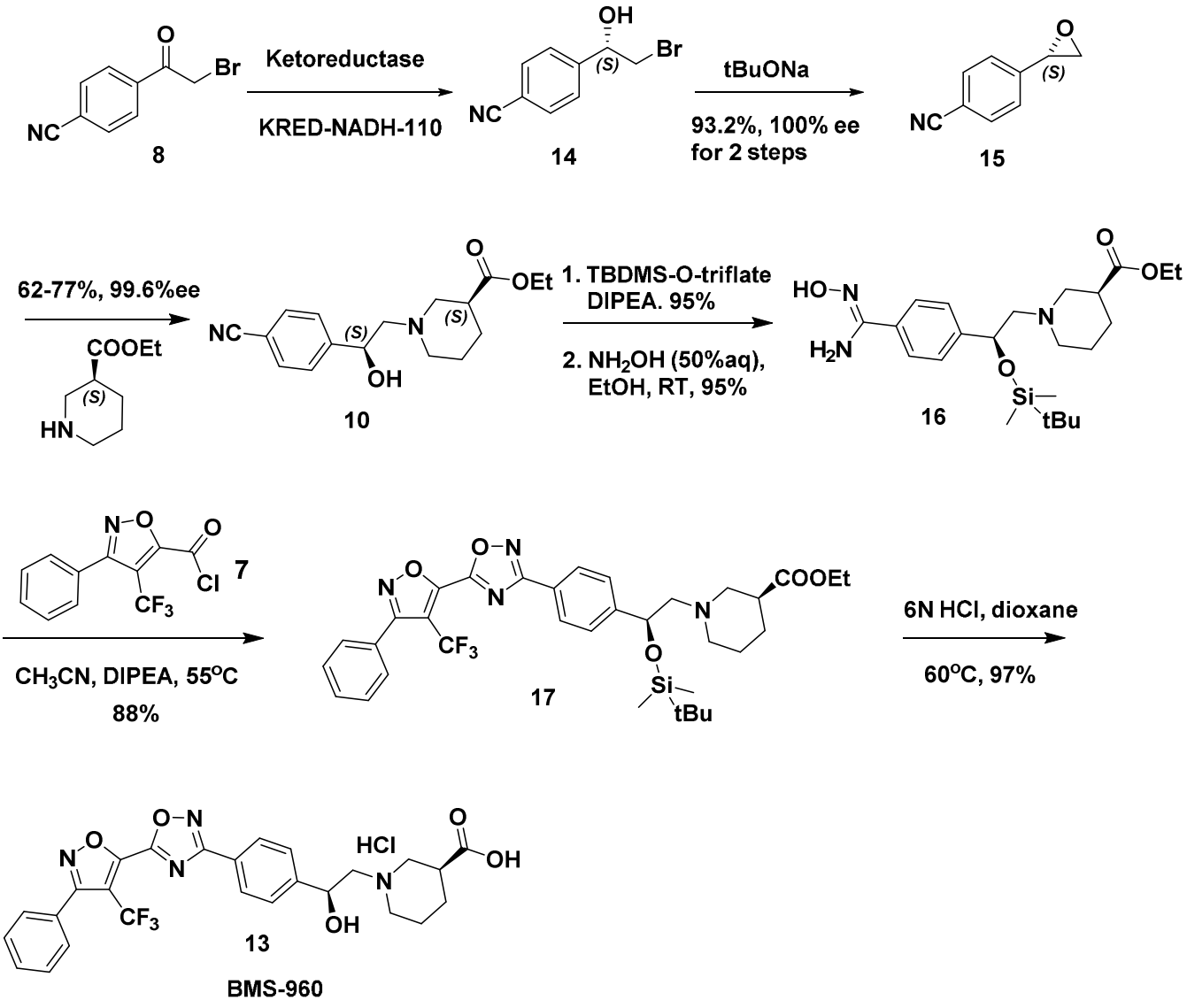
Scheme 6. Synthesis of the common intermediate **(15)**



With a synthesis of the chiral epoxide in-hand, our attention then turned to the subsequent ring-opening reaction needed to install the amino-ester portion of the molecule (Scheme 7). Under a

catalytic DMAP condition at 50 C, this proceeded as a highly regioselective and stereosepecfic process to afford **10** which was successfully recrystallized to give the amino alcohol with high ee and chemical purity. The secondary alcohol was now protected with a TBDMS group and as anticipated, the oxadiazole formation yield was improved from 50% to 88% with this protecting group change. During amidooxime formation, hydroxyl amine HCl salt was replaced with hydroxyl amine aqueous free base to avoid the previously needed neutralization and IPA was replaced with EtOH at RT rather than reflux to avoid ester exchange. Under the above mild conditions, the amidooxime formation proceeded with 90% yield. During the oxadiazole formation, acyl chloride rather than acyl fluoride was prepared to avoid the corrosive reagent, cyanuric fluoride.

Scheme 7: Alternative Synthesis of BMS-960



In conclusion, a practical enzymatic reduction to asymmetrically reduce the bromoketone **8** was developed. Furthermore, a novel regioselective and stereospecific epoxide ring opening procedure was established. The improved process enabled us to efficiently prepare multiple batches of BMS-960 for toxicological studies including Ames testing.

EXPERIMENTAL SECTION

All reagents were obtained from commercial sources and used without further purification unless otherwise stated. All reactions were carried out under a nitrogen atmosphere. All glassware was dried and purged with nitrogen before use. Unless otherwise stated, all reactions were monitored by Shimadzu LCMS system using the following method: Phenomenex C18 column 10 μm 4.6 X 50 mm. Solvent: A = 10% methanol/90% water with 0.1% TFA; B = 90% methanol/10% water with 0.1% TFA. Gradient: 0-100% B over 4 min. Flow: 4 mL/min, Wavelength: 220 nm. HPLC method-1 was used for achiral analysis of enzymatic reactions and performed on a Phenomenex Synergi MAX-RP column (4 μm, 50 × 4.6 mm) at ambient temperature with a flow rate of 2 mL/min and a gradient of solvent A (0.01 M NH_4OAc in CH_3CN-Water 5:95) and solvent B (0.01 M NH_4OAc in CH_3CN -Water 95:5) from 30% to 60% solvent B over 3 min and holding at 60% B for an additional 2 min. The detection was by UV at 220 nm. The retention times were 1.7 min for **14** or **18**, 1.9 min for **15** or its *(R)*-isomer**,** and 2.7 min for **8**. HPLC method-2 was used for chiral separation of **14** and **18** and was performed on a Chiralpak AS-RH column (5 μm, 150 × 4.6 mm) with a flow rate of 0.5 mL/min at ambient temperature and a gradient of Solvent A (0.01 M NH_4OAc in CH_3CN-Water 10:90) and Solvent B (0.01 M NH_4OAc in CH_3CN-Water 90:10) holding at 35% solvent B for the first 12 min, then from 35% to 60% solvent B over 3 min. The detection was by UV at 220nm. The retention times were 9.1 min for **14**, 11.4 min for **18.** HPLC method-3 was used for chiral separation of **15** and its *(R)*-isomer and was performed on a Chiralpak AD-RH column (5 μm, 150 × 4.6 mm) with a flow rate of 0.5 mL/min at ambient temperature

and a gradient of Solvent A (0.01 M NH_4OAc in CH_3CN-Water 10:90) and Solvent B (0.01 M NH_4OAc in CH_3CN-Water 90:10) holding at 35% solvent B for the first 12 min, then from 35% to 80% solvent B over 3 min, and holding at 80% B for additional 5 min. The detection was by UV at 220nm. The retention times were 16.7 min for **15**, 14.0 min for its *(R)*-isomer. All ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-d_6 or $CDCl_3$ as the solvents unless otherwise specified. Codexis ketoreductase KRED-NADH-110 (Lot# 2011608WW, 25 U/mg), Glucose dehydrogenase (GDH-102, Lot# 011005CL, 90 U/mg) and NAD (Free acid, MW 663.4, Boehringer Mannheim, Lot83431124-72, Feb/97), and the ketone 2-bromo-4'-cyanoacetophenone (CAS number 20099-89-2, from Aldrich, Lot 08528KH, chemical purity 96%) were used. The 0.2 M, pH 6.0 sodium phosphate buffer was prepared and the recipe for 1 L buffer is Na_2HPO_4 24.6 mmol, FW 141.96, 3.492 g; NaH_2PO_4 175.4 mmol, FW 119.98, 21.044 g and DI water 1 L.

(*S*)-4-(oxiran-2-yl)benzonitrile (15). To a 5-L jacketed reactor were added 2 L of sodium phosphate buffer (0.2 M, pH 6.0), 96 g of D-glucose (1.2 equivalents), 4 g of NAD (β-Nicotinamide adenine dinucleotide), 8 kU of GDH (Glucose dehydrogenase), 500 mg of ketoreductase KRED-NADH-110, followed by 0.5 L of additional sodium phosphate buffer (0.2 M, pH 6.0) to rinse the different containers. The mixture was stirred to dissolve all solids and showed a pH of 5.5, which was adjusted to 6.0 with 1 M NaOH (About 90 mL). After 30min, a solution of 2-bromo-4'-cyanoacetophenone (**8)** (100 g, 446 mmol) in 250 mL DMSO was added, followed by an additional 50 mL DMSO, which was used to rinse the container. The mixture was stirred at 40 °C throughout the reaction (5 h). The pH was maintained between 5.5 and 6.1 with 1 M NaOH (total consumption about 450 mL). The mixture was extracted with 2 L of MTBE. The aqueous layer was extracted a second time with 1.6 L of MTBE. The combined MTBE extracts was washed with 0.5 L of 25% brine. The crude bromohydrin **14** solution was treated with sodium *tert*-butoxide solid (103 g, 2.4 equivalents, portionwise) at ambient temperature for 1 h until no bromohydrin **14** remained (checked by HPLC). The mixture was filtered through a pad of Celite. The cake was washed twice with 100 mL of MTBE. The

combined filtrate and MTBE washing was washed with 0.5 L of sodium phosphate buffer (0.2 M, pH 6.0) and with 0.5 L of 25% brine. Removal of solvent from the MTBE solution on a rotary evaporator at 30 °C gave a solid, which was transferred into a dish and further dried in a vacuum oven at ambient temperature for 3 days to give 60.4 g of the (*S*)-epoxide **15** as a yellowish solid, yield 93.2%, AP 99.9%, ee 100%. ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.75 - 7.58 (m, 2H), 7.47 - 7.35 (m, 2H), 3.91 (dd, *J*=4.0, 2.4 Hz, 1H), 3.20 (dd, *J*=5.5, 4.0 Hz, 1H), 2.76 (dd, *J*=5.6, 2.5 Hz, 1H); MS *m/e* 146.1($M+H^+$); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 1.16 minutes.

(*S*)-ethyl 1-((S)-2-(4-cyanophenyl)-2-hydroxyethyl)piperidine-3-carboxylate (10). (*S*)-4-(oxiran-2-yl)benzonitrile (10.0 g, 68.9 mmol) was dissolved in IPA (100 mL), 4-Dimethylaminopyridine (1.683 g, 13.78 mmol) was added, followed by (*S*)-ethyl piperidine-3-carboxylate (10.8 g, 68.9 mmol). The reaction mixture was heated up to 50^{o}C overnight. The reaction mixture was then cooled to RT. EtOAc (200 ml) was added, followed by 80 ml of H_2O. The organic layer was separated and concentrated under vacuum to give (*S*)-ethyl 1-((*S*)-2-(4-cyanophenyl)-2-hydroxyethyl)piperidine-3-carboxylate (18.9 g, 59.9 mmol, 87 % yield). *Recrystallization* The crude product was recrystallized from EtOH / EtOAc / H_2O (3/2/2, v/v) (8ml / 1g crude) to give the desired product 15g (80% recovery yield, 99% chemical purity, 99.6% EE) as a crystalline off-white solid. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.69 - 7.59 (m, 2H), 7.50 (d, *J*=7.9 Hz, 2H), 4.78 (dd, *J*=10.6, 3.3 Hz, 1H), 4.18 (q, *J*=7.0 Hz, 3H), 3.14 (d, *J*=9.5 Hz, 1H), 2.70 - 2.52 (m, 3H), 2.52 - 2.30 (m, 3H), 2.04 - 1.87 (m, 1H), 1.83 - 1.54 (m, 3H), 1.29 (t, *J*=7.2 Hz, 3H); ^{13}C NMR (101MHz, $CDCl_3$) δ 173.7, 147.7, 132.2 (s, 2C), 126.4 (s, 2C), 118.8, 111.2, 68.4, 65.9, 60.6, 54.5, 54.4, 41.7, 26.6, 24.6, 14.2; MS *m/e* 303.1($M+H^+$); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 0.95 minutes.

(*S*)-ethyl 1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-cyanophenyl)ethyl) piperidine-3-

carboxylate. To a mixture of (*S*)-ethyl 1-((*S*)-2-(4-cyanophenyl)-2-hydroxyethyl)piperidine-3-carboxylate (17.00 g, 56.2 mmol) and DIPEA (17.68 ml, 101 mmol) in DCM (187 ml) was added tert-butyldimethylsilyl trifluoromethane-sulfonate (16 ml, 69.6 mmol) slowly. The reaction was complete in 2 hours by HPLC. The reaction mixture (a light brown solution) was quenched with ice water. The aqueous was extracted with DCM. Organic phase was combined, dried with Na_2SO_4. After concentration, the crude material was further purified by chromatography (330g silica, 10-30% ethyl acetate/hexane) to afford (*S*)-ethyl 1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-cyanophenyl)ethyl)piperidine-3-carboxylate (22.25 g, 53.4 mmol, 95 % yield) as a viscous film. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.60 (d, *J*=8.4 Hz, 2H), 7.46 (d, *J*=8.4 Hz, 2H), 4.78 (t, *J*=6.1 Hz, 1H), 4.22 - 4.05 (m, 2H), 2.91 - 2.83 (m, 1H), 2.81 - 2.73 (m, 1H), 2.59 (dd, *J*=13.3, 6.7 Hz, 1H), 2.53 - 2.44 (m, 1H), 2.39 (dd, *J*=13.2, 5.5 Hz, 1H), 2.36 - 2.30 (m, 1H), 2.19 - 2.08 (m, 1H), 1.96 - 1.84 (m, 1H), 1.74 - 1.63 (m, 1H), 1.53 - 1.38 (m, 2H), 1.25 (t, *J*=7.2 Hz, 3H), 0.89 (s, 9H), 0.09 (s, 3H), -0.07 (s, 3H); HRMS (ESI) *m*/*e* 417.25765 [$(M + H)^+$, calcd for $C_{23}H_{37}N_2O_3Si$ 417.25680]. HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 3.05 minutes.

(*S*)-ethyl 1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-((Z)-N'-hydroxycarbamimidoyl)phenyl) ethyl)piperidine-3-carboxylate (16). (*S*)-ethyl-1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-cyanophenyl)ethyl)piperidine-3-carboxylate (31.0 g, 74.4 mmol) was dissolved in EtOH (248 ml). Hydroxylamine (50% aq.) (6.84 ml, 112 mmol) was added and stirred at RT overnight. Then, all volatiles were removed in vacuum. The residue was purified by chromatography (330g silica, 0% - 50% ethyl acetate/hexane). (*S*)-ethyl-1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-((*Z*)-N'-hydroxycarbamimidoyl) phenyl) ethyl)piperidine-3-carboxylate (31 g, 68.9 mmol, 93 % yield) was obtained as a white foam. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.39 (d, *J*=4.6 Hz, 1H), 7.57 (d, *J*=8.4 Hz, 2H), 7.36 (d, *J*=8.1 Hz, 2H), 4.88 (br. s., 2H), 4.84 - 4.76 (m, 1H), 4.18 - 4.06 (m, 2H), 2.97 (dd, *J*=11.1, 3.9 Hz, 1H), 2.83 (dt, *J*=7.4, 3.9 Hz, 1H), 2.61 (dd, *J*=13.4, 7.3 Hz, 1H), 2.57 - 2.48 (m, 1H), 2.42 (dd, *J*=13.2, 4.6 Hz, 1H), 2.30 (t, *J*=10.7 Hz, 1H), 2.17 - 2.07 (m, 1H), 1.97 - 1.87 (m, 1H), 1.73 - 1.63 (m,

1H), 1.62 - 1.50 (m, 1H), 1.48 - 1.34 (m, 1H), 1.25 (t, *J*=7.2 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 3H), -0.09 (s, 3H); ^{13}C NMR (101MHz, $CDCl_3$) δ 174.3, 152.6, 146.5, 131.1, 126.4, 125.4, 73.0, 68.0, 60.3, 56.2, 54.7, 42.0, 26.8, 25.9 (s, 3C), 24.7, 18.3, 14.2, 0.0, -4.7 (d, *J*=4.6 Hz, 2C); HRMS (ESI) *m*/*e* 450.27922 [$(M + H)^+$, calcd for $C_{23}H_{40}O_4N_3Si$ 450.27826]; HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 2.25 minutes.

3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid (3). Ethyl 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylate (40g, 140 mmol) was dissolved in THF (240 mL) and water (24.01 mL), then cooled to 0°C (internal temperature). Subsequently, LiOH monohydrate (5.04 g, 210 mmol) was added in portions and monitored with HPLC. The reaction mixture was stirred overnight at 0^{o}C. HPLC showed 17% AP of starting material. Then, 0.1eq (336mg) of LiOH was added at 0^{o}C and gradually warmed up to RT for 3 hours. The reaction mixture was re-cooled to 0^{o}C, then diluted with 200mL ether and 200mL water. The aqueous phase was separated and washed with ether (2X50mL). The organics were combined and extracted with water (3X30mL). The aqueous phases were combined, cooled to 0^{o}C, and then neutralized with 1N HCl and extracted with diethyl ether (3x200mL). Ether layers were combined and dried with Na_2SO_4. After concentration, 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid (29.5 g, 114 mmol, 81 % yield) was obtained as pale solid (purity 99%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.44 - 7.68 (m, 5H), 6.2-7.0 (br, 1H); MS *m/e* 257.8($M+H^+$); HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 2.45 minutes.

3-Phenyl-4-(trifluoromethyl)isoxazole-5-carbonyl chloride (7). 3-phenyl-4-(trifluoromethyl)isoxazole-5-carboxylic acid (29.2g, 114 mmol) was suspended in thionyl chloride (100 ml, 1370 mmol) at RT. Catalytic amount of DMF (a few drops) was added. The reaction mixture was heated up to 90^{o}C for 24 hours. The reaction was monitored with HPLC (Note: all HPLC samples were

prepared by quenching an aliquot of reaction mixture with anhydrous methanol and monitoring for the resulting methyl ester). HPLC at 24 hour indicated that the reaction was completed (peak to peak conversion). The reaction mixture was cooled down to RT diluted with 100mL anhydrous toluene, then concentrated in vacuum. The yellow oil residue was further pumped overnight under high vacuum to afford a gray solid (31.3g, 114mmol, 100% yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.50 - 7.63 (m, 5H). HPLC (XBridge 5µ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 3.29 minutes (methyl ester derivatized from MeOH quench).

(*S*)-ethyl 1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylate (penultimate) (17) (*S*)-ethyl 1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-((*Z*)-N'-hydroxycarbamimidoyl)phenyl)ethyl)piperidine-3-carboxylate (32.6g, 72.5 mmol) was dissolved in anhydrous acetonitrile (145 ml) and cooled to ~3^{o}C with ice bath. 3-phenyl-4-(trifluoromethyl)isoxazole-5-carbonyl chloride (19.98 g, 72.5 mmol) was dissolved in 50mL anhydrous acetonitrile and added dropwise. The internal temperature was kept below 10^{o}C during addition. Once addition was completed, the reaction mixture was naturally warmed up to RT. At 30 minutes, HPLC showed the 1st step was complete. The reaction mixture was re-cooled to below 10^{o}C. DIPEA (18.99 ml, 109 mmol) was added slowly. After the addition, the reaction mixture was heated up to 55^{o}C for 17 hours. HPLC/LCMS showed a complete conversion. All volatiles were removed in vacuum. The residue was stirred in 250mL 20% ethyl acetate / hexane, DIPEA HCl salt was precipitated out, which was removed via filtration. The filtrate was concentrated and purified by chromatography (3 X 330g silica, 0% to 50% ethyl acetate / hexane). (*S*)-ethyl 1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylate (43g, 64.1 mmol, 88 % yield) was obtained a light yellow oil. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.15 (d, *J*=8.4 Hz, 2H), 7.73 - 7.65 (m, 2H), 7.61 - 7.49 (m, 5H), 4.88 - 4.81 (m, 1H), 4.19 - 4.06 (m, 2H), 2.97 (dd,

J=11.0, 3.7 Hz, 1H), 2.83 (d, *J*=11.4 Hz, 1H), 2.65 (dd, *J*=13.4, 7.0 Hz, 1H), 2.57 - 2.42 (m, 2H), 2.39 - 2.28 (m, 1H), 2.22 - 2.10 (m, 1H), 1.98 - 1.88 (m, 1H), 1.75 - 1.64 (m, 1H), 1.62 - 1.58 (m, 1H), 1.56 - 1.37 (m, 1H), 1.28 - 1.22 (m, 3H), 0.92 (s, 9H), 0.11 (s, 3H), -0.04 (s, 3H); ^{13}C NMR (101MHz, $CDCl_3$) δ 174.2, 169.6, 163.7, 162.1, 148.9, 131.0, 129.31, 128.62, 127.5, 126.9, 125.9, 124.1, 73.0, 67.9, 60.3, 56.3, 54.7, 42.1, 26.8, 25.9, 24.8, 18.3, 14.2, 0.0, -4.7; ^{19}F NMR (376MHz, $CDCl_3$) δ -55.04; HRMS (ESI) *m*/*e* 671.28867 [$(M + H)^+$, calcd for $C_{34}H_{42}O_5N_4F_3Si$ 671.28711]; HPLC (XBridge 5μ C18 4.6x50 mm, 4 mL/min, Solvent A: 10 % MeOH/water with 0.2 % H_3PO_4, Solvent B: 90 % MeOH/water with 0.2 % H_3PO_4, gradient with 0-100 % B over 4 minutes): 4.07 minutes

(*S*)-1-((*S*)-2-hydroxy-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylic acid, HCl (13, BMS-960) (*S*)-ethyl 1-((*S*)-2-(tert-butyldimethylsilyloxy)-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylate (42g, 62.6 mmol) was dissolved in 1,4-dioxane (150 ml) and 6N HCl (150 ml) was added (during addition, some cloudiness was observed which quickly disappeared upon stirring. By the end of addition, the reaction mixture became cloudy again). The reaction mixture was heated to 65ºC for 6 hours, and EtOH was distilled out to push equilibrium forward. 1,4-Dioxane was removed in vacuum. The residue was re-dissolved in CH_3CN/water (1:1 v/v) and lyophilized overnight. The crude ~37g solid/foam was obtained with ~3% impurity.

Recrystallization After lyophilization, ~37g light yellow solid/film was transferred to 2 liter three-necked flask with acetonitrile (740mL). The suspension was heated up to 60ºC, and then 15mL water was added gradually. A clear solution was observed, which was cooled to RT and concentrated to a small volume (very viscous but no precipitates). To the above viscous oil, 740mL ethyl acetate was added and vigorously stirred. A clear solution was observed. When heated up to 60ºC, the ethyl acetate solution gradually became cloudy, then precipitates were observed. The suspension was cooled to RT for 1 hour. The solid was collected and dried to afford (*S*)-1-((*S*)-2-hydroxy-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxylic acid, HCl (BMS-960) (30g, 87% yield, > 99% EE, 99.5% purity, no single impurity >0.5%). ^{1}H NMR (400MHz,

DMSO-d_6) δ 12.88 (br. s, 1H), 10.5 (br. s, 1H), 8.14 (d, *J*=8.6 Hz, 2H), 7.72 (d, *J*=8.4 Hz, 2H), 7.69 - 7.57 (m, 5H), 6.43 (br. s., 1H), 5.37 (d, *J*=10.8 Hz, 1H), 3.89 - 3.60 (m, 2H), 3.50 - 2.82 (m, 6H), 2.14 - 1.99 (m, 1H), 1.97 - 1.75 (m, 1H), 1.63 - 1.35 (m, 1H); ^{13}C NMR (101MHz, $CDCl_3$) δ 172.8, 168.5, 164.0, 161.6, 155.4, 156.2, 131.2, 129.0, 128.9, 127.4, 127.2, 125.5, 124.3, 120.2, 111.6, 66.6, 63.0, 52.9, 52.2, 38.8, 25.0, 21.7; ^{19}F NMR (376MHz, DMSO-d_6) δ -54.16; Anal. calcd for $C_{26}H_{23}F_3N_4O_5$.HCl: C, 54.71; H, 4.36; N, 9.80. Found: C, 54.76; H, 3.94; N, 9.76; HRMS (ESI) *m*/*e* 529.17040 [$(M + H)^+$, calcd for $C_{26}H_{24}N_4O_5F_3$ 529.16933].

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