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Practical synthesis of capromorelin, a growth hormone secretagogue, via a crystallization-induced dynamic resolution

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Abstract

A practical synthesis of capromorelin (**1**), a growth hormone secretagogue, is described that utilizes as a key step a crystallization-induced dynamic resolution (CIDR) of (±)-3a-benzyl-2-methyl-4,5,6,7-tetrahydro-2H-pyrazolo[4,3-c]pyridin-3(3aH)-one [(±)-**2**] by (*L*)-tartaric acid salt formation, yielding (R)-**2.*L*-tartaric acid** in high chemical yield (>85%) and with diastereomeric excess (de) of ~98%. Treatment of (R)-**2.*L*-tartaric acid** with ammonium hydroxide provided (R)-**2** without loss of chiral purity. In situ generated (R)-**2** was coupled with (R)-3-(benzyloxy)-2-(2-(tert-butoxycarbonyl)-2-methylpropanamido)propanoic acid [(R)-**3**] to give predominantly a single diastereomer of N-Boc-protected capromorelin [(1R,3aR)-**4**]. This process was used to prepare bulk quantities of capromorelin from (±)-**2** to support preclinical toxicology studies.

Keywords

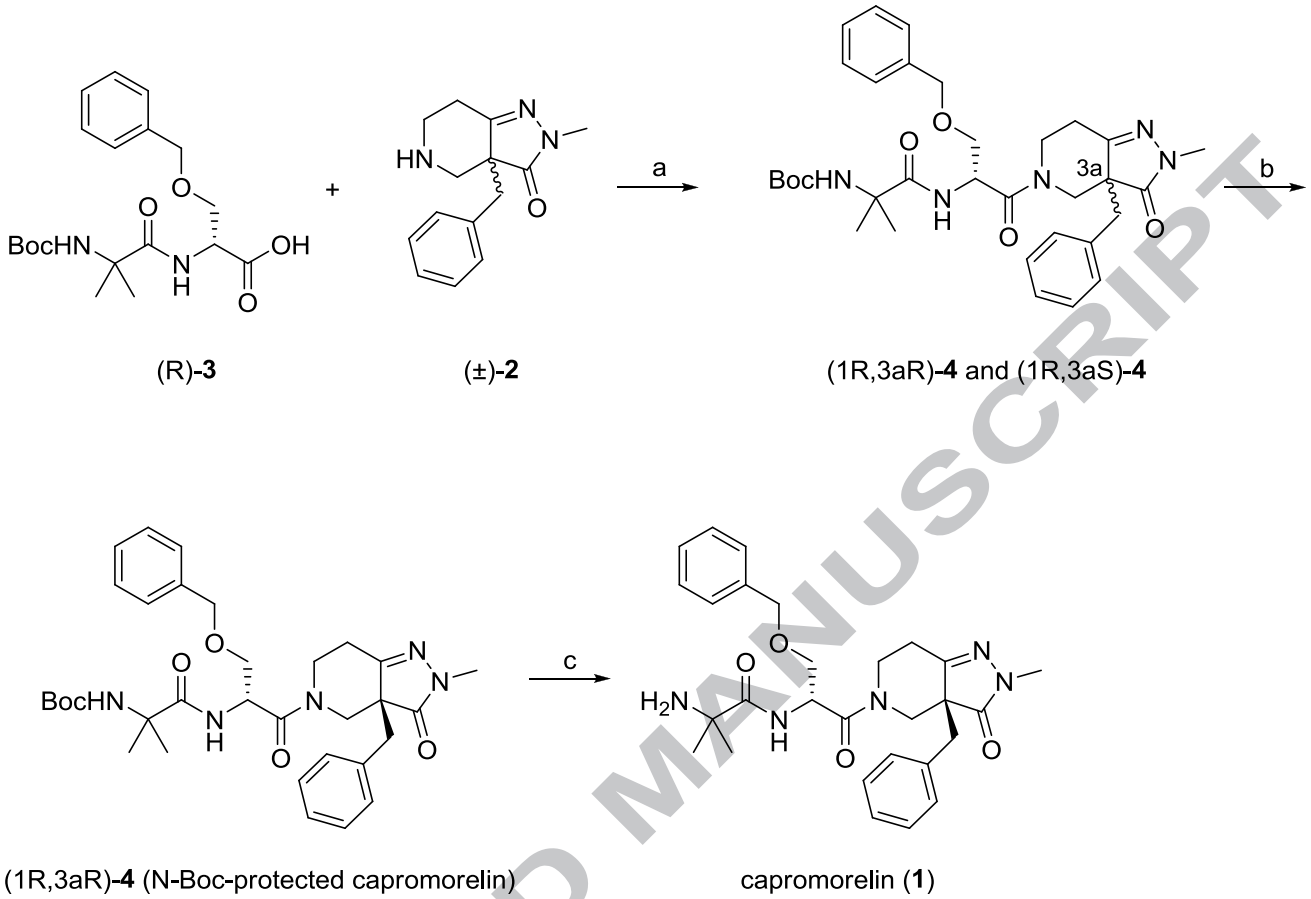
Capromorelin
Crystallization-induced dynamic resolution
Growth hormone secretagogue
GHS-R1a
Ghrelin mimetic

1. Introduction

Capromorelin (2-amino-N-[(1R)-2-[(3aR)-2,3,3a,4,6,7-hexahydro-2-methyl-3-oxo-3a-(phenylmethyl)-5H-pyrazolo[4,3-c]pyridin-5-yl]-2-oxo-1-[(phenylmethoxy)methyl]ethyl]-2-methyl-propanamide; **1**) is an orally active growth hormone secretagogue and small molecule mimetic of ghrelin, a hormone that plays a critical role in the regulation of energy homeostasis.[1] Capromorelin (in the form of its (*L*)-tartrate salt) was initially studied in human clinical trials as a treatment for frailty in elderly adults. Only modest improvements in various measures of physical performance were observed after one year in a Phase II clinical trial, insufficient to justify further clinical investment.[2]

Capromorelin was later investigated in companion animals as an orexigenic agent and was recently approved in 2016 by the Food and Drug Administration's Center for Veterinary Medicine as a treatment for inappetence in dogs with underlying pathophysiology that results in eating disorders (Entyce®).

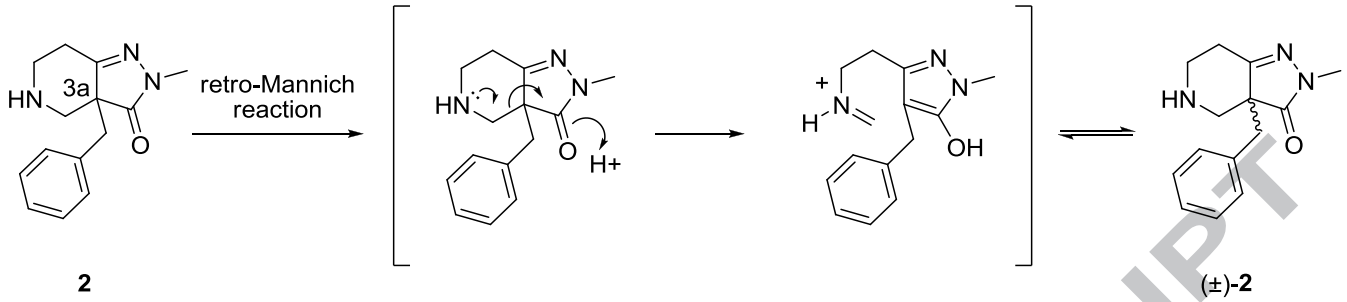
In the original discovery synthesis of capromorelin, the piperidine heterocycle, (±)-3a-benzyl-2-methyl-4,5,6,7-tetrahydro-2H-pyrazolo[4,3-c]pyridin-3(3aH)-one [(±)-**2**], prepared in a straightforward manner, was coupled with (R)-3-(benzyloxy)-2-(2-(tert-butoxycarbonyl)-2-methylpropanamido)propanoic acid [(R)-**3**] in the presence of triethylamine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and 1-hydroxy-7-azabenzotriazole (HOAt)[3] to yield a 1:1 mixture of two diastereomeric products, (1R,3aR)-**4** and (1R,3aS)-**4**, which were separated by silica gel column chromatography (see Scheme 1).[1] The desired diastereomer, (1R,3aR)-**4**, was treated with trifluoroacetic acid to remove the N-Boc protecting group and provide capromorelin of high diastereomeric excess (de). While this route could be used to prepare small scale batches of drug to support in vitro and in vivo pharmacology studies, it was not practical for synthesizing the larger amounts of drug needed for toxicology and clinical studies. Herein, we describe the development of a novel resolution of (±)-**2** by (*L*)-tartaric acid salt formation, yielding a single diastereomeric product, (R)-**2.*L*-tartaric acid**, in high chemical yield. We also report conditions for coupling (R)-**3** with enantiomerically pure (R)-**2**, generated in situ from (R)-**2.*L*-tartaric acid**, to give N-Boc-protected capromorelin, (1R,3aR)-**4**, without any need for chromatography.



Scheme 1. Discovery synthesis of capromorelin (**1**) starting from (±)-**2**. Reagents: (a) EDC, NEt_3, HOAt; (b) silica gel column chromatography; (c) trifluoroacetic acid.

2. Results and Discussion

A diastereomeric crystallization of (±)-**2** was initially investigated using several different chiral acids as resolving agents, the most effective of which was *L*-tartaric acid. Treatment of (±)-**2** with *L*-tartaric acid in wet acetone at room temperature provided an insoluble salt containing **2** of modest ~44% enantiomeric excess (ee). Interestingly, compound **2** obtained from the salt complex that remained behind in the acetone mother liquor was racemic, showing no enrichment of the opposite enantiomer as was expected. The racemization of **2** was proposed to proceed via a retro-Mannich reaction (Scheme 2). A survey of the literature revealed several examples of C3-carboxy or C3-cyano substituted piperidine derivatives that were found to epimerize via retro-Mannich reactions.[4]



Scheme 2. Proposed mechanism for the racemization of enantiomerically enriched **2** (stereochemistry undefined) that remained in solution during the diastereomeric crystallization process.

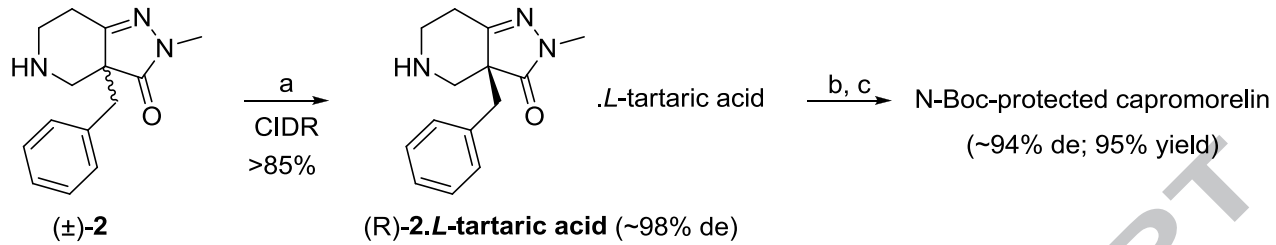
The facile racemization of 3a-benzyl-2-methyl-4,5,6,7-tetrahydro-2H-pyrazolo[4,3-c]pyridin-3(3aH)-ones such as **2** suggested the possibility of developing a dynamic kinetic resolution of (±)-**2**. In our work exploring diastereomeric crystallizations of (±)-**2**, we discovered that one of the diastereomeric *L*-tartrate salts of **2** was significantly less soluble in wet acetone compared to the other diastereomeric salt form and could be used to remove one of the enantiomers of **2** that remained in the acetone solution, potentially enabling a variation of a dynamic kinetic resolution called a crystallization-induced dynamic resolution (CIDR). Starting with the protocol used in the diastereomeric crystallization and experimenting with different combinations of acetone and water mixtures, we eventually found conditions under which a CIDR of (±)-**2** by chiral (*L*)-tartrate salt formation proceeded in high yield. A mixture of (±)-**2** and *L*-tartaric acid in acetone/water (25:1 ratio) was stirred at 50 °C for 70 h, providing an insoluble *L*-tartrate salt of **2** with diastereomeric excess (de) of ~98% and in >85% chemical yield, significantly greater than the maximum theoretical yield of a classical chiral salt crystallization (Fig. S1-2). The stereochemical configuration at the C3a position in **2.(*L*)-tartaric acid** obtained from the CIDR of (±)-**2** was assigned by first treating the compound with triethylamine to give the free amine **2**. Compound **2** was then coupled with (R)-**3** in the presence of EDC, HOAt, and triethylamine as previously described[1] to yield predominantly a product that exactly matched the ^{1}H spectral characteristics and thin layer chromatography profile of N-Boc-protected capromorelin (Fig. S3), thereby revealing the configuration of the C3a stereocenter in **2.(*L*)-tartaric acid** to be R. The configuration of the C3a stereocenter in N-Boc-protected capromorelin was established by x-ray crystallographic analysis of capromorelin (Fig. S4). Elemental analysis of (R)-**2.*L*-tartaric acid** prepared via a CIDR showed that a monotartrate 1.5 hydrate was formed. The CIDR of (±)-**2** was shown to be water sensitive as overall yields of the diastereomeric salt product significantly decreased if dry acetone was used as a solvent instead of an acetone:water mixture. The resolution of (±)-**2** was

successfully scaled up from 1 to >100 g to give (R)-**2.*L*-tartaric acid** without loss of chiral purity. Stability studies conducted on (R)-**2.*L*-tartaric acid** did not reveal any significant issues or loss of de upon standing for several months. In contrast, the free amine, (R)-**2**, was shown to readily racemize upon standing at room temperature or at 0 °C as an amorphous solid.

With an efficient CIDR process in hand to prepare multi-gram quantities of (R)-**2.*L*-tartaric acid**, we next turned our attention to the bulk synthesis of N-Boc-protected capromorelin. Since (R)-**2** was found to undergo partial racemization when stored as a solid or when generated on scale-up in the presence of triethylamine at 0 °C, we developed a protocol for the in situ generation of (R)-**2**. A slurry of (R)-**2.*L*-tartaric acid** in CH_2Cl_2 was treated at 0 °C with 2 equivalents of NH_4OH, yielding (R)-**2** of >98 ee (Fig. S5) and an insoluble ammonium tartrate salt which was removed by filtration to minimize concerns that it might promote epimerization of (R)-**2**. Stability studies showed that a CH_2Cl_2 solution of (R)-**2** could be stored for at least 1 h at 0 °C without (R)-**2** undergoing any significant epimerization. The CH_2Cl_2 solution containing (R)-**2** was subsequently treated at 0 °C with (R)-**3** in the presence of EDC and HOAt, providing N-Boc-protected capromorelin of ~94% de and in 95% chemical yield (Scheme 3; Fig S6-7). A minor amount of epimerization of (R)-**2** was found to occur during the coupling reaction. The salt exchange of (R)-**2.L-tartaric acid** with NH_4OH and the coupling of (R)-**2** with (R)-**3** were scaled from 1 to >100 g to give N-Boc-protected capromorelin without any significant loss of diastereomeric purity or other issues. N-Boc-protected capromorelin was converted into capromorelin as previously described, at which point the small amounts of the undesired capromorelin diastereomers that formed during the key coupling reaction could be removed.[1]

3. Conclusions

A novel CIDR of (±)-**2** by chiral (*L*)-tartrate salt formation was developed to provide (R)-**2.*L*-tartaric acid** in high yield and diastereomeric purity. An efficient and scalable salt exchange of (R)-**2.*L*-tartaric acid** with NH_4OH in CH_2Cl_2 provided (R)-**2** with minimal epimerization. In situ generated (R)-**2** was coupled with (R)-**3** in the presence of EDC and HOAt to give N-Boc-protected capromorelin of ~94% de. This process enabled the bulk preparation of the penultimate intermediate in the synthesis of capromorelin without a requirement for chromatography to separate diastereomers and facilitated the practical synthesis of bulk lots that were needed to support pre-clinical toxicology studies (Scheme 3).



Scheme 3. Practical synthesis of N-Boc-protected capromorelin from (±)-**2**. Reagents and conditions: (a) *L*-tartaric acid, 25:1 acetone:H_2O, 50 °C, 70 h (b) NH_4OH, CH_2Cl_2, 0 °C; (c) (R)-**3**, EDC, HOAt, CH_2Cl_2, 0 °C.

4. Experimental

4.1 Chemical Synthesis

^{1}H NMR spectra were recorded on either a Varian XL-300 or Varian VNMRS 600 spectrometer. Chemical shifts are expressed in ppm relative to internal $CDCl_3$ or CD_3OD. Low resolution mass spectra were obtained on either a Fisons PlatForm II mass spectrometer utilizing ApcI ionization or a Fisons Thermospray TSP1000 mass spectrometer. Solvents and reagents were commercially available and used directly without further purification. All reactions were monitored by thin-layer chromatography on 0.25 mm x 5 cm x 10 cm silica gel 60 GF-254 (EM Science) plates using ultraviolet light for visualization. Flash chromatography was performed using 40-63 μm silica gel (EM Science). HPLC methodology for ee determinations: Chiral Pak AS column, eluting with hexane/2-propanol (65:35) at a flow rate of 0.6 ml/min, UV detection at 231 nm; HPLC methodology for de determination: Chiral Tech IC 250 mm x 4.6 mm, 5 micron column using a gradient of CO_2/MeOH + 0.2% NH_3 in MeOH.

4.1.1 Synthesis of 3aR-benzyl-2-methyl-4,5,6,7-tetrahydro-2H-pyrazolo[4,3-c]pyridin-3(3aH)-one.*L*-tartrate [(R)-2.*L*-tartaric acid]

A mixture (±)-**2** (5.000 g, 20.6 mmol) and *L*-tartaric acid (3.092 g, 20.6 mmol) in acetone (80 mL) and H_2O (3.2 mL) was heated at 50 °C under N_2 for 70 h, during which time the reaction mixture became a thick suspension. The reaction mixture was slowly cooled to 23 °C and filtered to give a solid that was washed with acetone (3 x 50 mL) and dried *in vacuo* to give 7.034 g (87% yield) of (R)-**2.*L*-tartaric acid** as a white solid. An aliquot of the *L*-tartaric acid salt was treated with triethylamine in CH_2Cl_2 at room temperature to provide (R)-**2** which was subjected to chiral HPLC analysis for ee determination (98% ee; Fig. S1-2). ^{1}H NMR (CD_3OD) δ 7.20 – 7.19 (m, 3H), 7.08 – 7.07 (d, J = 6.2 Hz, 2H), 4.48 (s, 2H), 3.63 – 3.60 (m, 1H), 3.54 – 3.43 (m, 2H), 3.30 – 3.28 (m, 2H), 3.07 (d, J = 13.5 Hz, 1H), 2.92 – 2.87 (m, 2H), 2.86 – 2.78 (m, 1H), 2.63 (d, 13.1 Hz, 1H); MS m/e 244 (M^++1).

Elemental anal. calcd for $C_{18}H_{23}N_3O_7 \cdot 1.5H_2O$: C, 51.42; H, 6.23; N, 10.00. Found: C, 51.19; H, 6.04; N, 9.97.

4.1.2 Synthesis of 3aR-benzyl-2-methyl-4,5,6,7-tetrahydro-2H-pyrazolo[4,3-c]pyridin-3(3aH)-one [(R)-2]

Concentrated NH_4OH (0.34 mL, 5.1 mmol) was added to a suspension of (R)-**2.*L*-tartaric acid** (1.00 g, 2.5 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 0.25 h and filtered. The filtrate containing free amine (R)-**2** (>98% ee; Fig. S5) was used immediately in the coupling reaction.

4.1.3 Synthesis of {1-[2-(3aR-Benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(R)-benzyloxymethyl-2-oxo-ethylcarbamoyl]-1-methyl-ethyl}-carbamic acid tert-butyl ester [(1R,3aR)-4]

A mixture of (R)-**2** in CH_2Cl_2 from above [assumed to contain 3.09 g, 12.7 mmol of (R)-**2**], (R)-**3** (4.84 g, 12.7 mmol), EDC (2.45 g, 12.8 mmol), and HOAt (2.60 g, 19.1 mmol) was stirred at 0 °C for 1 h, then was warmed to 23 °C and allowed to stir for 16 h. The reaction mixture was filtered, and the filtrate was washed with saturated aq. $NaHCO_3$ (100 mL) and H_2O (100 mL), dried over $MgSO_4$, and evaporated *in vacuo* to give 7.35 g (95% yield) of (1R,3aR)-**4** as a white solid (94% de; Fig. S6-7). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.07 (m, 12H), 5.26 (d, J = 6.46 Hz, 1H), 5.07 (d, J = 12.91 Hz, 1H), 4.83 (br. s., 1H), 4.50 (d, J = 7.04 Hz, 2H), 3.72 (m, 1H), 3.58 – 3.67 (m, 1H), 3.16 (d, J = 13.50 Hz, 1H), 3.07 (s, 3H), 2.83 – 2.95 (m, 3H), 2.56 (d, J = 9.98 Hz, 1H), 2.49 (d, J = 12.91 Hz, 1H), 1.47 (d, J = 19.37 Hz, 6H), 1.32 – 1.42 (m, 9H) (Fig. S8); MS m/e 606 (M^++1).

4.1.4 Synthesis of capromorelin (1) for x-ray crystallographic analysis

A solution of (1R,3aR)-**4** (64.7 g, 106.8 mmol) in CH_2Cl_2 (300 mL) at 0 °C was treated slowly with ice-cold trifluoroacetic acid (300 mL) over 1.5 h. The resulting mixture was warmed to room temperature, stirred for an additional 3 h, and filtered to yield a clear yellow solution which was concentrated and re-dissolved in ethyl acetate. The ethyl acetate solution was washed with saturated aq. $NaHCO_3$ (3x) and saturated aq. NaCl (2x), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 49 g of an orange foam. A small aliquot of the orange foam was dissolved in isopropyl alcohol and stirred overnight at 50 °C to give white crystals of capromorelin which were removed by filtration and found suitable for x-ray crystallography studies. The remaining foam was fully characterized after it was transformed into capromorelin.(*L*)-tartaric acid as previously described.[1]

4.2 X-ray crystallography studies

A representative crystal of capromorelin was surveyed and a 1 Å data set was collected on a Siemens R3RA/v diffractometer. Atomic scattering factors were taken from the International Tables for X-ray Crystallography.[5] All crystallographic calculations were facilitated by the SHETXTL system.[6] All diffractometer data were collected at room temperature.

Structure summary for capromorelin (**1**): empirical formula = $C_{28}H_{35}N_5O_4$; formula weight = 505.6; calculated density (g/cm^3) = 1.184; color, habit = colorless prismatic; crystal size (mm) = 0.16 x 0.22 x 0.44; crystal system = monoclinic; space group = C2; unit cell dimensions a = 24.035 (2) Å, b = 8.0450 (10) Å, c = 15.7380 (10) Å, α = 90.0°, β = 111.180 (10)°; volume = 2837.6(5) Å^3; Z = 4; absorption coefficient (mm^{-1}) = 0.651; F(000) = 1080.

Coordinates and structural factors can be obtained free of charge from The Cambridge Crystallographic Data Centre (accession code: CCDC 1517678).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at:

Disclosures

C.R., B.L., A.W., and P.C. are employees of Pfizer, Inc.

References

1. Carpino, P. A.; Lefker, B. A.; Toler, S. M.; Pan, L. C.; Hadcock, J. R.; Cook, E. R.; DiBrino, J. N.; Campeta, A. M.; DeNinno, S. L.; Chidsey-Frink, K. L.; Hada, W. A.; Inthavongsay, J.; Mangano, F. M.; Mullins, M. A.; Nickerson, D. F.; Ng, O.; Pirie, C. M.; Ragan, J. A.; Rose, C. R.; Tess, D. A.; Wright, A. S.; Yu, L.; Zawistoski, M. P.; DaSilva-Jardine, P. A.; Wilson, T. C.; Thompson, D. D. *Bioorg. Med. Chem.* **2003,** *11*, 581.
2. White, H. K.; Petrie, C. D.; Landschulz, W.; MacLean, D.; Taylor, A.; Lyles, K.; Wei, J. Y.; Hoffman, A. R.; Salvatori, R.; Ettinger, M. P.; Morey, M. C.; Blackman, M. R.; Merriam, G. R. *J. Clin. Endocrinol. Metab.* **2009,** *94*, 1198.
3. Carpino, L. A. *J. Am. Chem. Soc.* **1993,** *115*, 4397.
4. Gmeiner, P.; Feldman, P. L.; Chu-Moyer, M. Y.; Rapoport, H. *J. Org. Chem.* **1990,** *55*, 3068.
5. Ibers, J. A.; Hamilton, W. C.; Editors, *International Tables for X-Ray Crystallography, Vol. 4: Revised and Supplementary Tables to Volumes 2 and 3*. Kynoch Press: 1974.
6. Sheldrick, G. M. *Acta Crystallogr., Sect. C: Struct. Chem.* **2015,** *71*, 3.

Graphical Abstract

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