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Development of a scalable synthesis of a nonbasic inhibitor of the serine protease tryptase

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Abstract—A chromatography-free process for the synthesis of a bis(benzimidazole)difluoromethane inhibitor of the serine protease tryptase is described. This synthesis features the introduction of the *gem*-difluoro moiety using the electrophilic fluorinating reagent *N*-fluoro-bis(phenylsulfonimide) as well as the stepwise introduction of both benzimidazole rings. A protocol for the destruction of reactive, process-related substances produced in the synthesis is also presented.

1. Introduction

Inhibition of mast cell tryptase has been recognized as a viable therapeutic target for allergic and inflammatory diseases such as allergic rhinitis, conjunctivitis, dermatitis, inflammatory bowel disease (IBD), and asthma.[1] For example, a Phase IIa clinical evaluation of the tryptase inhibitor APC-366 (**1**) showed significance in the primary endpoint of reduction of the late asthmatic response when administered by inhalation.[2] In addition, in a Phase II clinical trial with patients exhibiting mildly to moderately active ulcerative colitis, intravenous administration of APC-2059 (**2**) resulted in 58% of these patients experiencing a clinically significant benefit in symptoms associated with the disease.[3] Other tryptase inhibitors, either in pre-clinical or clinical development, include RWJ-56423 (**3**) and BMS-354326 (**4**). Structures for these compounds are shown in Figure 1.

Another class of tryptase inhibitors is based on the 2,2′-bis(benzimidazole)methane scaffold, including the symmetrical bis(5-amidino-2-benzimidazolyl)methane (BABIM; **5**). The BABIM series afforded one of the first potent inhibitors of mast cell tryptase, having been initially reported as a potent inhibitor of other serine proteases in the late 1970s.[4,5]

Research at Celera (formerly Axys Pharmaceuticals) led to the discovery of a novel binding mode of these inhibitors and determined that the potency of these compounds against trypsin-like proteases such as Factor Xa, and tryptase result from the tetrahedral binding of zinc(II) ions to both the hydroxyl group of Ser-195 and the imidazole moiety of His-57 in the active site of the enzyme, along with two nitrogens of the bis(benzimidazole)methane moiety of the inhibitor.[6] This novel mechanism takes advantage of ambient levels of zinc in vivo, especially in the granules of the mast cell. However, development of orally active BABIM derivatives as serine protease inhibitors, including tryptase, were hampered by the presence of the polar benzamidine and metabolically labile benzylamine P_1 moieties, illustrated in the BABIM class (**5**) as well as the unsymmetrical derivative, APD-10 (**6**). In order to identify an orally active tryptase inhibitor based on the bis(benzimidazole)methane motif, efforts were undertaken to identify related derivatives which did not rely on the benzamidine or similar polar functionality for binding affinity in the P_1 pocket of the enzyme. Ultimately, SAR efforts identified CRA-9249 (**7**) as an orally active tryptase inhibitor devoid of these polar functionalities at P_1 (Fig. 2). In order to provide multigram and ultimately larger quantities of **7** for pre-clinical evaluation, we set out to develop a scalable process for this compound.

2. Results and discussion

The initial route to CRA-9249 was based on a stepwise, 'one-pot' coupling of 2,2-difluoromalonic acid (**8**) with

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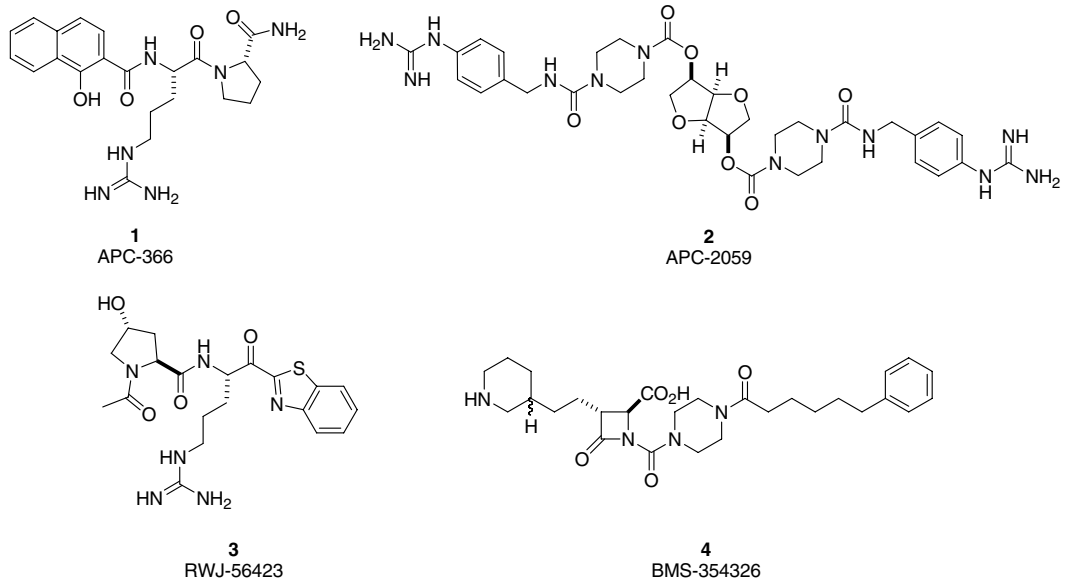


Figure 1. Representative tryptase inhibitors.

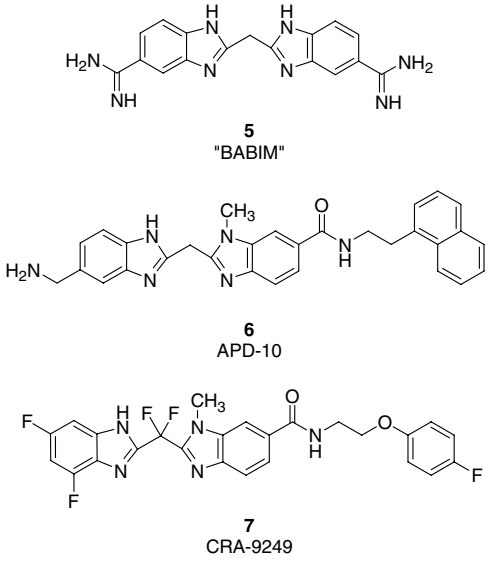
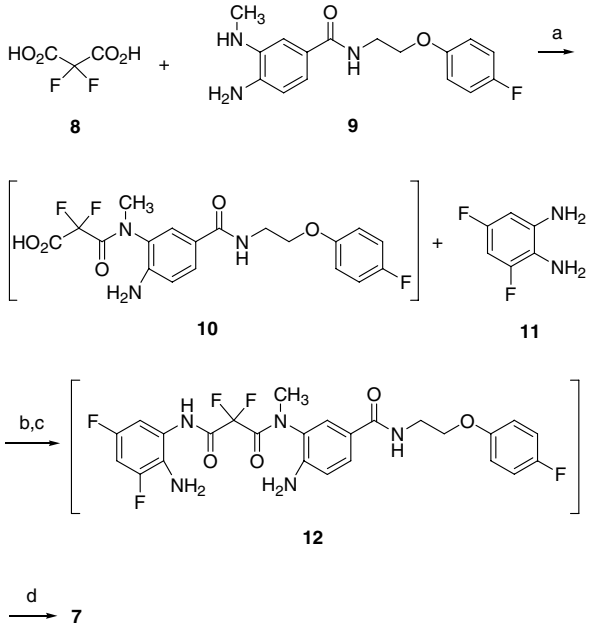


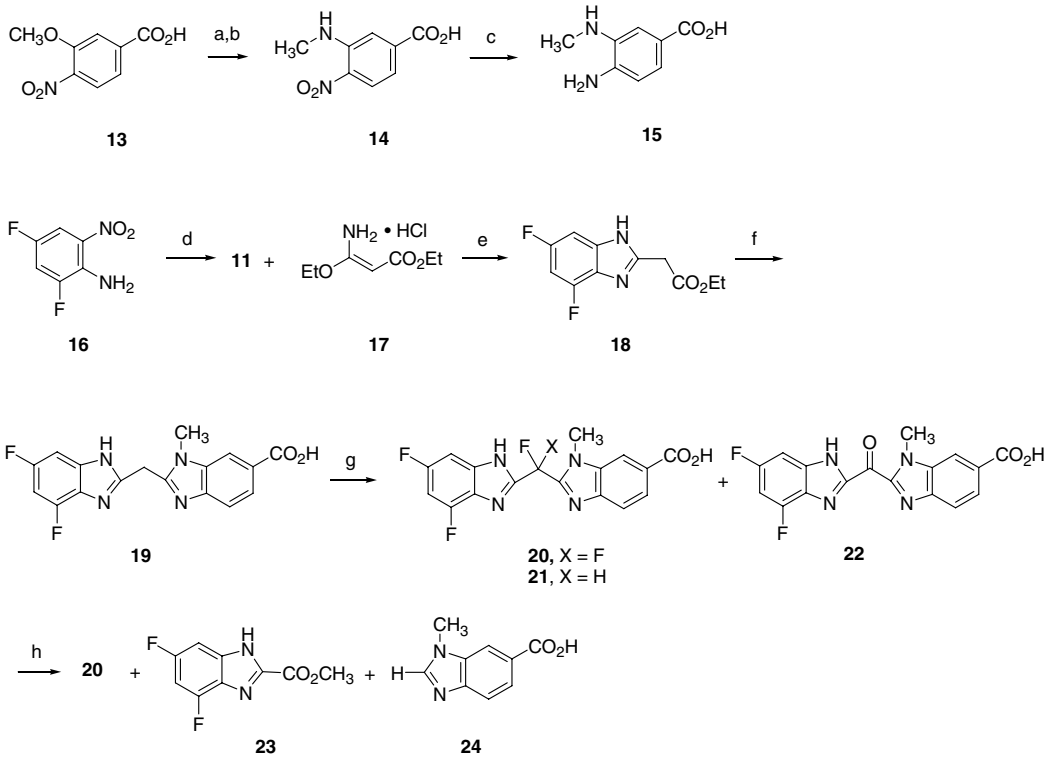
Figure 2. Bis(benzimidazole)methane derived tryptase inhibitors.

two different 1,2-diaminobenzene derivatives **9** and **11** to each carboxylate functionality of **8** with the expensive coupling agent bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP) to give the bis(amide) **12**.[7,8] Without isolation, compound **12** was heated in acetic acid to generate both benzimidazole rings of CRA-9249. Although this approach was satisfactory in providing modest amounts of **7** needed for early discovery efforts, the route suffers from low yield (20% from diacid **8** after column chromatography) and therefore was unsuitable for the manufacture of multigram to kilogram quantities of the active pharmaceutical ingredient (API, compound **7**).

In order to arrive at a scalable process to **7**, which circumvented the liabilities evident in the route described in Scheme 1, we pursued a stepwise condensation route to the bis(benzimidazole)methane fragment. Generation of the *gem*-difluoromethane bridgehead would rely on the reaction of the bridgehead methylene of the bis benzimidazole moiety with an electrophilic fluorinating reagent. This transformation is based upon a successful strategy previously employed at Celera on a similar bis(benzimidazole)methane derivative.[9] The synthesis of the key bis(benzimidazole)difluoromethane acid **20** is shown in Scheme 2. Both benzimidazole rings in **20** were constructed from two separate 1,2-diaminobenzene derivatives according to this scheme. First, the right-hand benzimidazole ring was constructed starting from



Scheme 1. Reagents and conditions: (a) PyBrOP, *N*-methylmorpholine (NMM), DMF, −10 °C to rt; (b) PyBrOP, DMF, −10 °C to rt; (c) **11**, NMM, −10 °C to rt; (d) AcOH, 85 °C, 3 h.



Scheme 2. Reagents and conditions: (a) 40% aq CH_3NH_2, 100–105 °C; (b) 1 N aq HCl, 75% for both steps; (c) H_2, 5% Pd–C, THF–MeOH (8:3), 96%; (d) H_2, 10% Pd–C, EtOH, 89%; (e) EtOH, rt to 35–40 °C; (f) **15**, DMPU, 150 °C, 90%; (g) $(PhSO_2)_2N$–F, 1,4-dioxane, reflux; 91%; (h) MeOH, reflux.

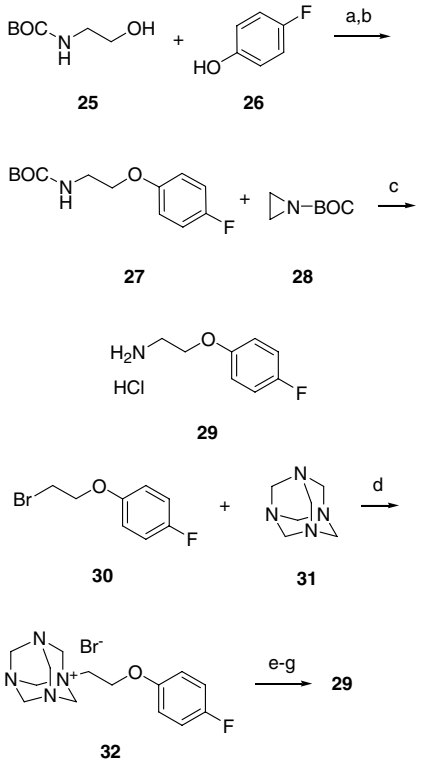
commercially available 3-methoxy-4-nitrobenzoic acid (**13**) which was converted to the 3-methylamino derivative **14** via a nucleophilic aromatic displacement of the methoxy group with 40% aqueous methylamine. Reduction of the nitro group in **14** with hydrogen and 5% palladium on carbon provided 4-amino-3-methylaminobenzoic acid **15**.[7]

The 3,5-difluoro-1,2-diaminobenzene required for the left-hand benzimidazole ring was produced by catalytic reduction of commercially available 3,5-difluoro-6-nitroaniline (**16**) with 10% Pd–C to afford the 3,5-difluoro-1,2-diaminobenzene (**11**). Reaction of the diamine **11** with the imidate of ethyl cyanoacetate (**17**)[10] in ethanol at 35–40 °C provided the left-hand benzimidazole fragment **18** in 65% yield. Finally, thermal condensation of ester **18** with a degassed suspension of 3-methylamino-4-methyl benzoic acid **15** in DMPU at 150 °C afforded the bis(benzimidazole)methanecarboxylic acid **19**.[11] Fluorination of this acid with an excess (250 mol %) of *N*-fluorobenzenesulfonimide afforded the desired difluoro bridgehead intermediate **20** in 74% yield after heating the crude product in refluxing methanol. The hot methanol treatment served to degrade small amounts of the monofluoro- and keto-derivatives **21** and **22** derived from incomplete fluorination and competitive air oxidation of the methylene group in **19**. Although we had determined that bis(benzimidazole)methyl derivatives similar to **19** were toxic, we suspected that the keto derivative **22**, as well as the corresponding amide related to **7**, could also be toxic. Furthermore, the chemical reactivity of **22** to hot methanol also supported our concerns that these ketones could also be toxic.

Hence, stringent specifications (<0.05%) for their presence were put in place for the final API. The treatment of crude **20** in refluxing methanol served to degrade the reactive monofluoro- and keto-derivatives **21** and **22** and reduce their levels below these stringent specifications prior to the introduction of the 2′-(4-fluorophenoxy)ethylamine fragment. The resulting degradants **23** and **24** were very soluble and easily removed during the methanol treatment.

Several routes were evaluated to prepare 4-fluorophenoxyethylamine derivative **29** required for the synthesis of CRA-9249 (**7**), which are described in Scheme 3. The first route started from the Mitsunobu reaction of commercially available *N*-(*tert*-butoxycarbonyl)-aminoethanol **25** and 4-fluorophenol (**26**) using diisopropylazodicarboxylate (DIAD) and triphenylphosphine to provide Boc-protected 2-phenoxyethylamine **27**. Unfortunately this approach was ultimately abandoned due the formation of modest amounts (15–20% as determined by proton NMR analysis) of the *N*-Boc aziridine (**28**), which required a tedious chromatographic separation to remove this potentially troublesome impurity from the desired *N*-Boc-aminophenoxyether.[12,13]

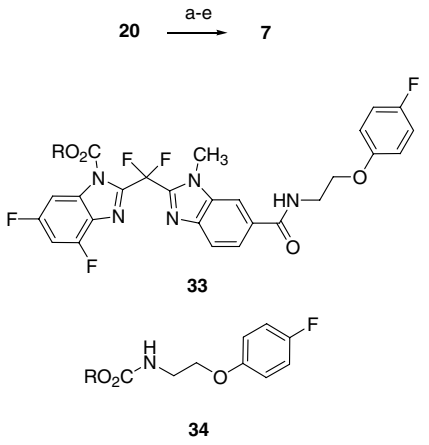
Although this route could be employed to afford the target amine hydrochloride **29** after deprotection with a solution of anhydrous HCl, a more scalable approach was developed, based on the Delepine reaction.[14] The



Scheme 3. Preparation of the right-hand amine **29**. Reagents and conditions: (a) Ph_3P, THF, rt to 0–5 °C; (b) *i*-$PrO_2CN{=}NCO_2Pr$-*i*, 0–5 °C, then 0–5 °C to rt; (c) silica gel chromatography then 4 N HCl in 1,4-dioxane, CH_2Cl_2, 0–5 °C to rt (60% from **25**); (d) $CHCl_3$, reflux, 95%; (e) EtOH, 12 N HCl; (f) 4 N aq NaOH, H_2O, 88% for two steps; (g) anhyd HCl in 1,4-dioxane, then Et_2O, 85%.

commercially available 4-fluorophenoxy-ethylbromide (**30**) was reacted with hexamethylenetetramine (**31**) to provide the ammonium salt **32**. Hydrolysis of **32** to the free amine followed by conversion to the HCl salt provided amine **29** on a multigram scale without chromatography. Several other routes were explored but none were as convenient as the Delepine reaction for large-scale preparations of amine **29**.[15]

The coupling of amine **29** and acid **20** using mixed anhydrides afforded very good to excellent yields after addition of water to crystallize the product from *N*,*N*-dimethylformamide (DMF). We noted that the nature of the chloroformate employed in the reaction made a significant difference in conversion and yield of the crude product. Use of either isobutyl chloroformate (R = *i*-Bu) or isopropyl chloroformate (R = *i*-Pr) afforded CRA-9249 (**7**) in excellent purity. However isopropyl chloroformate was found to be superior to isobutyl chloroformate in the conversion of **20** to **7**. The latter reagent provided as much as 18% of unreacted **20** which fortunately could be easily recovered through acidification of the filtrate. Attempts to convert unreacted acid **20** through charging additional chloroformate to the reaction mixture resulted in the formation of side-products, such as compound **33** which is produced by N-acylation of the free N–H of the left-hand benzimidazole nitrogen and carbamate **34** which arises from competitive reaction of excess chloroformate with amine **30**. Thus, 1 M



Scheme 4. Reagents and conditions: (a) DMF, NMM, 0–5 °C; (b) ROCOCl, (R = *i*-Pr or *i*-Bu), <10 °C; (c) **29**, DMF; (d) NMM, <10 °C to rt; (e) recrystallization from ethanol.

isopropyl chloroformate in toluene was the preferred reagent to generate the mixed anhydride, ensuring >95% conversion and excellent yields of CRA-9249 (Scheme 4).

CRA-9249 (**7**) was isolated in 72% yield after chloroformate-mediated amide bond formation and recrystallization of the crude solid. Ultimately, this process was used to generate over 95 g of **7** in >99.0% purity. The limit of detection for the keto- and monofluoro impurities was less than 0.03% as determined by spiking experiments with authentic samples. The only impurity identified at significant levels (>0.1%) was the acid precursor **20** (0.25%).[16]

Acknowledgments

We thank Joyce Mordenti, Mike Green, and Mike Venuti for their commitment to and guidance during the execution of this work. In addition Celera would like to acknowledge the support and contributions of Bayer A.G. in the identification of CRA-9249.

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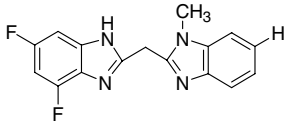
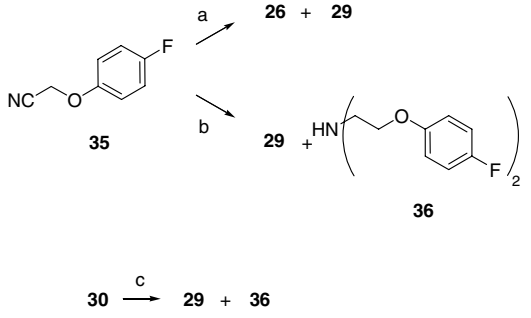
8. Although bis(amide) **12** was not isolated, this intermediate could be produced as a mixture of regioisomers. The specific regioisomer shown here is based on the assumption that acylation occurs on the more basic nitrogens found on each 1,2-diaminobenzene derivative **9** and **11**.
9. Sperandio, D.; Gangloff, A. R.; Litvak, J.; Goldsmith, R.; Hataye, J. M.; Wang, V. R.; Shelton, E. J.; Elrod, K.; Janc, J. W.; Clark, J. M.; Rice, K.; Weinheimer, S.; Yeung, K.-S.; Meanwell, N. A.; Hernandez, D.; Staab, A. J.; Venables, B. L.; Spencer, J. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3129–3133.
10. Imidate **17** was prepared in 85% yield on a 1.1 mol scale from the reaction of ethyl cyanoacetate and HCl gas in a mixture of toluene and ethanol (~6:1) at 0–5 °C.
11. The conditions used to produce **19** also afforded small amounts of the decarboxylated bis(benzimidazole)methane derivative shown below, whose identity was confirmed by comparison to an authentic sample.



12. The preparation of compound **29** by alkylation of α-chloroacetamide with 4-fluorophenol followed by $LiAlH_4$ reduction has been described in the literature: Shtacher, G.; Taub, W. *J. Med. Chem.* **1966**, *9*, 197–203. However, we did not evaluate this process for the work described here.
13. Protection of commercially available 2-bromoethylamine hydrobromide with di-*tert*-butyl dicarboxylate and *N*-methylmorpholine (NMM), followed by bromide displacement with 4-fluorophenol and cesium carbonate also provided **27** contaminated with aziridine **28**.
14. (a) Galat, A.; Elion, G. *J. Am. Chem. Soc.* **1939**, *61*, 3585–3586. For a review of synthetic applications of hexamethylenetetramine, including the Delépine reaction, please see the following: (b) Blažević, N.; Kolbah, D.; Belin, B.; Šunjić, V.; Kajfež, F. *Synthesis* **1979**, 161–176.
15. Other routes explored in the preparation of **29** included reduction of nitrile **35** and reaction of bromide **30** with ammonia. Reaction of **35** using either catalytic [e.g., H_2/PtO_2; transformation (a)] or chemical reduction [e.g., BH_3·THF or $LiAlH_4$, THF, reflux; transformation (b)] were unsatisfactory, providing either C–O bond cleavage as the primary reaction pathway or formation of small amounts of *N*,*N*-bis[2-(4′-fluorophenoxy)ethyl]amine (**36**). The hydrogenolysis of the C–O bond of α-phenoxyacetonitriles is a known process: Benarab, A.; Boyé, S.; Savelon, L.; Guillaumet, G. *Tetrahedron Lett.* **1993**, *34*, 7567–7568, Displacement of the bromide in **30** with ammonia in several solvent combinations and conditions (transformation c) was also plagued by formation of **36** and the corresponding trialkylamine which could not be easily separated from the desired product.



16. The 95-g lot of **7** also provided acceptable analysis results including combustion (CHN), inductively coupled plasma (ICP), residue on ignition (ROI) and moisture content (Karl Fischer).
LC-MS: *m/z* 516.0 $[M+1]^+$; ^1H NMR (DMSO-d_6; 400 MHz) δ 8.8 (t, 1H), 8.3 (s, 1H), 7.85 (d, 1H), 7.8 (d, 1H), 7.3 (d, 1H), 7.2 (t, 1H), 7.1 (m, 2H), 7.0 (m, 2H), 4.1 (t, 2H), 4.0 (s, 3H), 3.7 (q, 2H); CHN: calcd: C, 58.26; H, 3.52; N, 13.59; F, 18.43. Found: C, 58.03; H, 3.28; N, 13.62; F, 18.55; ICP results: Zn: <1 ppm; Cu < 1 ppm. ROI: <0.10%; % moisture (Karl Fischer): <0.10%.