Synthesis of Novel Tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridines via Intramolecular Nitrilimine Cycloaddition

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The preparation of novel tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridines is reported. Pivotal to the synthesis of these compounds was the development of mild reaction conditions to generate a highly functionalized nitrilimine capable of undergoing an intramolecular cycloaddition with a tethered alkyne. The desired cycloadduct was formed as an equal mixture of diastereomers.

Key words intramolecular cycloaddition; nitrilimine; pyrazole; γ -secretase inhibitor

Alzheimer's disease (AD) is the most prevalent form of senile dementia affecting the elderly.¹⁾ A diagnostic hallmark of AD is the presence of neuritic plaques composed of aggregated amyloid β -peptides (A β). This observation has led to the amyloid cascade hypothesis which asserts that accumulated A β in the brain is responsible for the neurodegeneration associated with the disease.²⁾ A β peptides are derived from the sequential cleavage of the transmembrane amyloid precursor protein (APP) by β -secretase and γ -secretase. Thus, γ -secretase inhibitors offer the potential as disease modifying agents for the treatment of AD.³⁾ In addition to processing APP, γ -secretase is also responsible for the cleavage of a number of other transmembrane proteins including Notch. Notch cleavage by γ -secretase releases the Notch intracellular domain (NICD) and following translocation to the nucleus the NICD serves as a transcription cofactor.⁴⁾ Since it has been well established that inhibition of the Notch signaling pathway leads to gastrointestinal and immune system related toxicities, a goal of this research was to identify an APP selective γ -secretase inhibitor.^{5,6)}

We recently reported the discovery of tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridines, including **1**, as a novel class of γ -secretase inhibitors^{7,8)} (Fig. 1). Compound **1** is a highly potent and metabolically stable inhibitor which demonstrated robust *in vivo* activity. Additionally, **1** exhibited 69-fold APP selectivity in a cellular based assay.⁹⁾ Because efforts in this series suggested replacement of the C6 cyclopropyl group with halophenyl functionality might well impart cellular selectivity of greater than 200-fold, we were motivated to prepare analogs **2a** and **2b**.

Results and Discussion

Chemistry Initially, we attempted to apply the methods developed for synthesis of **1** to the syntheses of **2a** and **2b**. Key to the preparation of **1** was the intramolecular cycloaddition of diazoketone **3** to yield ketopyrazole **4** followed by treatment with neat bis-(2-methoxyethyl)aminosulfur trifluoride (BAST) (Chart 1). Unfortunately, attempts to prepare ketopyrazole **6** failed to give any of the desired product but

instead yielded pyrazolopyridine **8** (Chart 2). The isolation of **8** can be explained by the decomposition of **6** *via* loss of sulfonic acid followed by tautomerization of **7** to observed product **8**. Having encountered this result, an alternate synthetic approach was investigated.

Intramolecular 1,3-dipolar cycloaddition of nitrilimines with alkynes is a well documented method for the preparation of fused polycyclic pyrazoles.^{10,11)} While nitrilimines have been obtained by a number of means including the thermolysis of 2,5-disubstituted tetrazoles¹²⁾ or 1,3,4-oxadiazolin-5-ones,¹³⁾ the photolysis of sydnones,¹⁴⁾ and the oxidation of aldehyde hydrazones,¹⁵⁾ they are most conveniently derived from the reaction of hydrazonoyl chlorides with base or silver salts.¹⁶⁾ This approach required the preparation of acyl hydrazide **16** as illustrated in Chart 3. The synthesis of α,α -difluoro- β -amino ester **12** was modified from methodology reported by workers at Merck.¹⁷⁾ Thus, the Ti(OEt)₄ mediated condensation of (*S*)-*t*-butylsulfonamide with 4-chlorobenzaldehyde gave sulfonimine **10**. Stereoselective addition of the Reformatsky reagent derived from ethyl bromodifluoroacetate to **10** gave propanoate **11** in a diastereomeric ratio of 9:1 as determined by ¹H-NMR. Following removal of the chiral auxiliary under acidic conditions, the resulting hydrochloride **12** was smoothly converted to sulfonamide **13**. Mitsunobu coupling with the appropriate racemic propargyl alcohol gave ester **14** as a 1:1 mixture of diastereomers. Subsequent hydrazinolysis and tosylation afforded the requisite acyl hydrazides **16**.

Chlorination of **16** with mesyl chloride in the presence of Hünig's base gave the desired hydrazonoyl chlorides **17** (Chart 4). The reaction mixture was then treated with additional base, in an attempt to generate the nitrilimine, but failed to

Fig. 1. Tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridines

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Reagents and conditions: (a) EtOH, 80°C; (b) BAST, 80°C.

Chart 1

Reagents and conditions: (a) EtOH, 80°C.

Chart 2

Fig. 2. ORTEP Diagram of 23

Reagents and conditions: (a) (*S*)-*t*-butylsulfonamide, $\text{Ti}(\text{OEt})_4$, THF, 89%; (b) $\text{BrCF}_2\text{CO}_2\text{Et}$, Zn powder, sonication, THF; (c) 4N HCl, dioxane–EtOH, 71% 2 steps; (d) 3-chlorosulfonyl-6-trifluoromethylpyridine, pyridine, 88%; (e) 1-cyclopropylprop-2-yn-1-ol, PPh_3 , DIAD, THF, 57%; (f) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, EtOH, quant.; (g) TsCl, pyridine, 50°C, 45%.

Chart 3

yield any of cycloadduct **18**. Treating **17** with Hünig's base in refluxing toluene gave an unidentifiable mixture of products. Recognizing the stability of a highly functionalized nitrilimine at elevated temperature might be problematic, a different approach was explored to promote nitrilimine formation and cycloaddition under mild reaction conditions. Hydrazide **16** was treated with triflic anhydride in the presence of excess 2,6-lutidine in CH_2Cl_2 at -15°C . Analysis of the reaction mixture by LC/MS suggested inner salt **19** had been formed. Formation of inner salts similar to **19**, from hydrazonoyl chlorides and pyridines, has been reported.¹⁸⁾

Since the formation of **19** could reasonably be assumed to

be the result of attack of lutidine on the triflate or the nitrilimine, the use of a more sterically hindered base was warranted. The reaction was conducted as before but utilizing 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as the base (Chart 5). Gratifyingly, LC/MS analysis of the reaction mixture showed two products with the desired mass, consistent with cycloaddition of isomeric nitrilimine, with either configuration at the propargyl amine stereocenter. Careful silica gel chromatography achieved separation of the diastereomeric cycloadducts **18** and **20**. Finally, removal of the tosyl group with hydrazine yielded pyrazoles **2a** and **21**. Utilizing the same methodology, pyrazoles **2b** and **25** were prepared from hydrazide **22**.

Reagents and conditions: (a) MsCl , $i\text{-Pr}_2\text{NEt}$, THF, 60°C ; (b) $i\text{-Pr}_2\text{NEt}$, THF, 60°C ; (c) $i\text{-Pr}_2\text{NEt}$, toluene, reflux; (d) Ti_2O , 2,6-lutidine, CH_2Cl_2 , -15°C .

Chart 4

Reagents and conditions: (a) Ti_2O , DTBMP, CH_2Cl_2 , -15°C , **18**, 18%; **20**, 24%; **23**, 11%; **24**, 12%; (b) NH_2NH_2 , EtOH, **2a**, 65%; **21**, 47%; **2b**, 72%; **25**, 75%.

Chart 5

The *syn* configuration of **23** was determined by X-ray crystallography¹⁹ (Fig. 2). This result provided for the stereochemical assignment of **24**, **2b** and **25**. Analysis of the ^1H -NMR data for compounds **23** and **24** revealed unique coupling data for the C-6 methine protons. For compound **23**, a doublet ($J=14.7\text{Hz}$) was observed at 5.75 ppm, while for **24** a doublet of doublets ($J=7.3$, 9.6Hz) was observed at 5.91 ppm. Similar data was obtained for compounds **18** and **20**, respectively. Thus, the combination of NMR data and the X-ray crystal result allowed for the assignment of *syn* stereochemistry to compounds **18** and **2a** and *anti* stereochemistry to compounds **20** and **21**. Compounds **2a** and **2b** were evaluated in the cellular selectively assay and unfortunately possessed APP selectivity of less than 100-fold.

Conclusion

In summary, we have described the preparation of novel tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridines *via* the intramolecular cycloaddition of a nitrilimine with a tethered alkyne. Generating the nitrilimine directly from a hydrazide at low temperature, using the very powerful dehydrating agent triflic anhydride and the very non-nucleophilic base DTBMP, was a key to success. This work is a further example of the powerful synthetic utility of dipolar cycloaddition in the construction of heterocyclic ring systems.

Experimental

General Flash chromatography was performed on a Teledyne Isco CombiFlash *Rf* automated chromatography system. ^1H - (400 MHz) and ^{13}C - (100 MHz) NMR spectra were

obtained on a Bruker Avance spectrometer using tetramethylsilane as internal standard with chemical shifts reported in ppm. LC/MS were recorded on an Agilent 1100-1946D LC-MS system (electrospray ionization (ESI), 3000V) employing a Phenomenex Luna C18 column (30×4.60mm). High resolution mass spectra were obtained by Professor Chad Nelson, Mass Spectrometry and Proteomics Facility, University of Utah utilizing a Thermo Electron LTQ-FT instrument (ESI, spray voltage, 2.8kV; heated capillary voltage, 120V). X-Ray crystallographic analysis was performed by Dr. Hu Pan, Department of Molecular Design, Élan Pharmaceuticals. The cellular selectivity assay was performed by Lany Ruslim, Department of Biology, Élan Pharmaceuticals.

Preparation of **23 and **24**** To 1.63 g **22** and 4.96 g 2,6-di-*tert*-butyl-4-methyl-pyridine in 60mL CH₂Cl₂ at –15°C was added dropwise, with stirring, a solution of triflic anhydride (2.73 g) in 10mL CH₂Cl₂. The resulting mixture was stirred at –15°C for 2h, and then the reaction was quenched by addition of 100mL of half-saturated aqueous NaHCO₃. The two phases were stirred together vigorously, and then the CH₂Cl₂ layer was separated. The aqueous layer was then extracted twice with CH₂Cl₂. The combined CH₂Cl₂ extracts were dried over Na₂SO₄, filtered, and evaporated. Flash chromatography of the residue (120g silica; 0–15% EtOAc–hexane) afforded 184mg (11%) of **23** and 200mg (12%) of **24**. For **23**: *R*_f=0.65 (9:1 hexane–ethyl acetate on silica); ¹H-NMR (CDCl₃) δ: 0.04–0.11 (1H, m), 0.27–0.40 (2H, m), 0.47–0.56 (1H, m), 0.88–0.96 (1H, m), 2.50 (3H, s), 4.21 (1H, d, *J*=9.7Hz), 5.75 (1H, d, *J*=14.7Hz), 6.95 (2H, t, *J*=8.6Hz), 7.25–7.31 (2H, m), 7.42 (2H, d, *J*=8.1Hz), 7.76 (1H, d, *J*=8.3Hz), 7.97 (2H, d, *J*=8.4Hz), 8.05 (1H, s), 8.28 (1H, d, *J*=8.2Hz), 8.97 (1H, d, *J*=1.8Hz); ¹³C-NMR (CDCl₃) δ: 5.5, 5.9, 19.3, 21.8, 57.8, 60.3 (t, *J*=29Hz), 115.6, 115.8, 120.6 (m), 121.1 (m), 120.6 (q, *J*=275Hz), 127.6, 128.7, 129.0 (m), 130.4, 130.8 (m), 132.7, 136.9 (d, *J*=3.4Hz), 139.7, 145.6 (dd, *J*=22.5, 28Hz), 147.1, 148.0, 151.0 (q, *J*=35.5Hz), 161.4, 163.9; High-resolution MS Calcd for C₂₈H₂₃F₆N₄O₄S₂ [M+H]⁺: 657.1067, Found: 657.1059. For **24**: *R*_f=0.50 (9:1 hexane–ethyl acetate on silica); ¹H-NMR (CDCl₃) δ: 0.31–0.44 (2H, m), 0.61–0.69 (1H, m), 0.70–0.85 (2H, m), 2.47 (3H, s), 4.11 (1H, d, *J*=8.6Hz), 5.91 (1H, dd, *J*=7.3, 9.6Hz), 6.86 (2H, t, *J*=8.6Hz), 7.13 (2H, dd, *J*=5.3, 8.5Hz), 7.37 (2H, d, *J*=8.3Hz), 7.73 (1H, d, *J*=8.3Hz), 7.91 (2H, d, *J*=8.3Hz), 8.04 (1H, s), 8.24 (1H, dd, *J*=2.0, 8.2Hz), 9.15 (1H, d, *J*=1.7Hz); ¹³C-NMR (CDCl₃) δ: 5.4, 9.7, 17.7, 22.2, 58.3, 63.5 (t, *J*=29Hz), 116.0, 116.2, 120.8, 121.5 (q, *J*=273Hz), 122.2 (m), 128.0, 128.9, 130.5, 131.1 (dd, *J*=2.1, 9.9Hz), 133.3, 136.7, 142.2, 146.7 (d, *J*=21.0Hz), 147.1 (d,

J=24.0Hz), 147.5, 148.6, 151.3 (q, *J*=35.0Hz), 161.9, 164.6; High-resolution MS Calcd for C₂₈H₂₃F₆N₄O₄S₂ [M+H]⁺: 657.1067, Found: 657.1059.

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- 19) For **23**: Crystal data: space group *P*2₁2₁2₁ (No. 19), *a*=14.132(2) Å, *b*=16.807(2) Å, *c*=25.556(4) Å, *V*=6070(15) Å³, *Z*=8, final *R* factors: *R*=0.0668, *R*_w=0.1741, and *R*₁=0.0592 for *I*>2.0σ(*I*) data. CCDC 847895 contains complete supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.