Tetrahedron Letters 61 (2020) 152352

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Facile synthesis of the glucosylceramide synthase inhibitor GZ667161

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ARTICLE INFO

Article history:
Received 12 March 2020
Revised 2 August 2020
Accepted 7 August 2020
Available online 16 August 2020

Keywords:
GZ667161
GZ-161
Glucosylceramide synthase
Gaucher disease
GBA mutations

ABSTRACT

GZ667161 (GZ-161) is a quinuclidine-based small molecule inhibitor of the lysosomal enzyme glucosylceramide synthase. It represents an important tool molecule for studying the contribution of glycosphingolipids to disease pathology in lysosomal storage disorders such as Gaucher disease and GBA1 Parkinson's disease. GZ667161 is not commercially available. The published synthesis involves 6 steps and proceeds in 18% overall reported yield. As part of a drug discovery program targeting Type 2 Gaucher disease we required quantities of GZ667161 that would support animal studies. To facilitate the project, we devised and executed an efficient 4-step convergent synthesis of the compound.

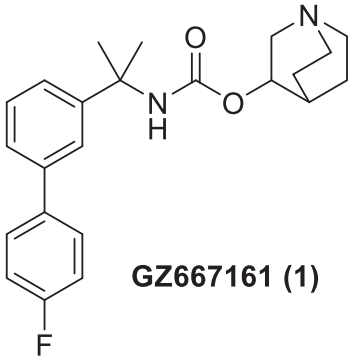
Introduction

Gaucher disease is the most common liposomal storage disorder, with a prevalence of 1 in 57,000 births [1]. Patients possess deleterious mutations in the *GBA1* gene encoding the lysosomal enzyme glucocerebrosidase (Gcase). Deficient or loss of Gcase activity results in accumulation of its substrates glucosylceramide and glucosylsphingosine in diseased tissues. Type 2 Gaucher disease is a variant that affects the neurological system, causing severe and irreversible brain damage during the first years of life. Additionally, accumulation of Gcase substrates in the brain stem may play a pathological role in the neurodegeneration associated with Parkinson's disease [2]. With no drugs approved for their treatment, Type 2 Gaucher disease and GBA-Parkinson's disease are highly unmet medical needs.

One of the current avenues under investigation for the treatment of Gaucher disease is preventing the synthesis of Gcase substrates. To accomplish this, the enzyme that produces glucosylceramide from ceramide, glucosylceramide synthase (GCS), has been targeted for inhibition. The quinuclidine analog GZ667161 (GZ-161, **1**, Fig. 1) [3] is an inhibitor of GCS. It has become an important tool molecule that is used to study the contribution of glycosphingolipids to disease pathology. The compound acts to reduce substrate flux into biosynthetic sphingolipid pathways. In a neuronopathic Gaucher disease mouse model, GZ667161 reduced brain glucosylceramide and glucosylsphingosine levels and significantly extended lifespan [4].

In a Gaucher-related synucleinopathy mouse model, GZ667161 also decreased α-synuclein and improved cognition in the mice [3]. Alpha-synuclein has also been implicated in the disease pathology of Parkinson's disease [5]. Recently, **1** has been shown to slow the accumulation of hippocampal aggregates of α-synuclein, ubiquitin, and tau, and improved the associated memory deficits [3].

As part of a drug discovery program targeting Type 2 Gaucher disease we required quantities of **1** to support *in vitro* and *in vivo*



GZ667161 (1)

Figure 1. Structure of GZ667161 (compound **1**) [4].

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https://doi.org/10.1016/j.tetlet.2020.152352
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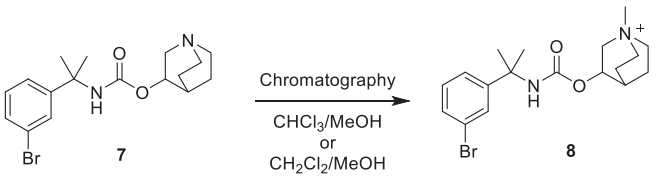
studies. The lack of a commercial supply of the molecule prompted us to synthesize it. The only published synthesis of GZ667161 appears in the patent literature [6,7]. However, in our hands, the published procedure failed to produce adequate quantities of **1** to meet our needs. We therefore designed and successfully carried out a modified synthesis of **1**. Our results are presented herein.

Results and discussion

The reported synthesis of **1** is shown in Scheme 1 [6] Treatment of methyl 3-bromobenzoate (**2**) with two equivalents of methyl magnesium iodide affords alcohol **3** in quantitative yield without purification. Alcohol **3** is then converted to chloroacetamide **4** *via* a Ritter reaction with chloroacetonitrile. Deacetylation of chloroacetamide **4** gives amine **5** (isolated as the hydrochloride salt). Oxalyl chloride is used to convert amine **5** to isocyanate **6** which is immediately converted to carbamate **7** through treatment with 3-quinuclidinol. Carbamate **7** is finally converted to **1** *via* a Suzuki reaction employing 4-fluorophenyl-boronic acid.

In our hands the Grignard addition and chloroacetamide formation/Ritter reaction proceeded smoothly and did not require purification. However, our ability to obtain amine **5** was severely hindered by difficulties in isolating the compound. Trituration as described in the literature afforded the intermediate, but, in a low yield that could not be improved (27% as opposed to 43% stated in the published synthesis). Purification of the subsequent carbamate **7** also proved to be problematic. The published synthesis cited the use of normal phase chromatography on silica gel with a mixture of chloroform and methanol with 2 N ammonium hydroxide as a modifier. In our hands, these conditions (and similar conditions substituting chloroform with dichloromethane) provided the quaternary salt **8** instead of the desired free base **7** (Scheme 2). We were finally able to obtain the hydrochloride salt of intermediate **7** in 74% yield through an ion exchange procedure in which the reaction mixture was first concentrated and the residuals were re-dissolved in 1 N aq. HCl to generate the water soluble quinuclidium salt. The aqueous solution was then washed with diethyl ether and ethyl acetate, and then concentrated. The resulting product was desalted by dissolving the residuals in isopropanol, followed by filtration. The filtrate was concentrated to give the hydrochloride salt of the desired product as a yellow powder.



Scheme 2. Attempts to purify intermediate **7** *via* chromatography resulting in the formation of quaternary by-product **8**.

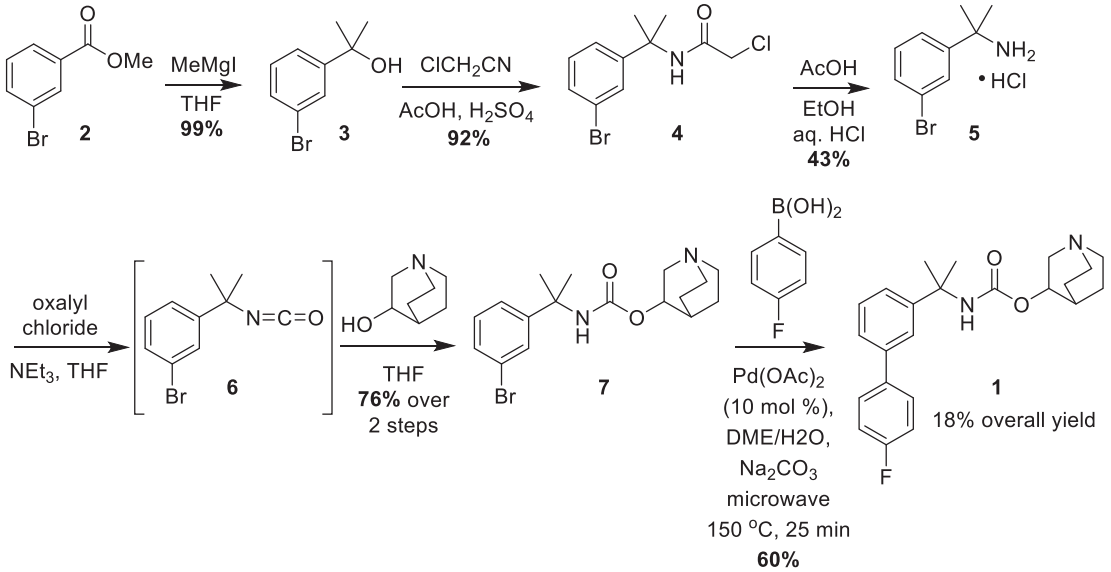
Unfortunately, we were unable to obtain the required quantities of the final target **1**, using the Suzuki chemistry methods described in the published method. Four different reaction conditions were explored (Table 1), however, we could not isolate the desired compound in appreciable quantities.

We hypothesized that the extremely nucleophilic quinuclidine nitrogen might be interfering with the reaction by associating with the palladium catalyst in preference to the boronic acid reagent (in a Buchwald/Hartwig fashion). In addition, the small amounts of crude **1** that were obtained (based on LC/MS) could not be purified adequately due to the co-elution of left over 4-fluorophenylboronic acid and the product under both normal phase and reversed-phase conditions. Reducing the amount of the boronic acid used had no effect on the ratio of product to 4-flurophenyl boronic acid at the completion of the reaction. We therefore designed an efficient four-step convergent synthesis that circumvented this problem by avoiding the use of the palladium catalyst in the presence of the nucleophilic quinuclidine nitrogen. Our modified synthesis is depicted in Scheme 3. The key to the modified synthetic sequence

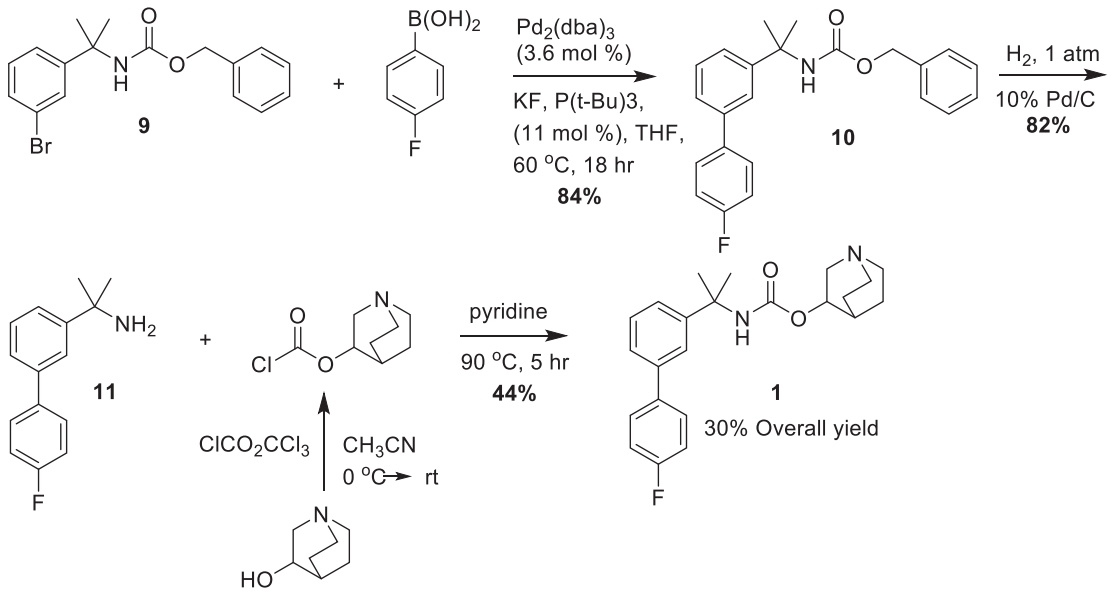
Table 1
Suzuki reaction conditions[a] explored for the attempted conversion of compound **7** to GZ667161 (**1**).

Catalyst	Additives	Solvent
[ab]$Pd(PPh_3)_4$ (10 mol %)	Na_2CO_3 (2 eq.)	Water/DME
$Pd(PPh_3)4$(10 mol %)	K_2CO3(2 eq.)	Water/DME
$Pd_2(dba)3$(10 mol %)	K_3PO_4, P(*cyclo*-C_6H_{11})3(2 eq.; 3 eq.)	Water/toluene
$Pd_2(dba)3$(10 mol %)	KF, P(*t*-Bu)3(1 eq.; 3 eq.)	THF

[a]Reactions were heated under microwave irradiation at 150 °C for 25 min
[b]Previously published conditions [6].



Scheme 1. Published synthesis of GZ667161 (compound **1**) [7].



Scheme 3. Modified 4-step convergent synthesis of GZ667161 (**1**).

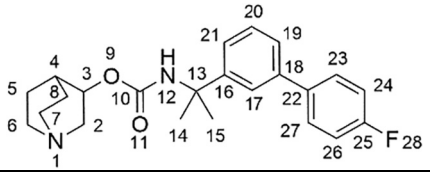
was to relocate the Suzuki step to the beginning of the synthesis and reserve insertion of the troublesome quinuclidine moiety until the end. Due to the previously describe purification challenges with amine **5**, commercially available benzyl (2-(3-bromophenyl) propan-2-yl)carbamate **9** was employed. Treatment of carbamate **9** with 4-fluorophenylboronic acid under typical Suzuki chemistry conditions afforded the desired 4-fluorophenyl derivative **10** in 84% yield following normal phase.

chromatography on silica gel. We did not require the use of microwave irradiation to obtain high yields of the product. The carbonylbenzyloxy (Cbz) group was then removed *via* catalytic hydrogenation at one atmosphere over 10% palladium on carbon to afford biphenyl amine **11** in 82% yield.

Difficulties obtaining an isocyanate derivate similar to intermediate **6** prompted the use of a convergent synthesis. 3-Quinuclidinol was first treated with diphosgene to provide quinuclidin-3-yl carbonochloridate hydrochloride (prepared as described in the literature and isolated as a white solid) [8]. Treatment of biphenyl amine **11** with the carbonochloridate in pyridine at 90 °C provided **1** as a white solid in 44% yield. Optimum purification conditions were found to be a basic amine column using a gradient of ethyl acetate in hexanes. Attempts to improve the yield of the final step of the synthesis proved unsuccessful. Nevertheless, we were able to reproducibly obtain **1** in 30% overall yield, which is higher than the yield reported for the published synthesis (18%).

The ^{1}H NMR spectra and ESIMS were consistent with that previously published [7]. Because of the broadness in the aliphatic ^{1}H NMR region and some protons in the aromatic rings, the ^{1}H NMR was run at different temperatures and in different solvents to enhance the resolution of the peaks, with the best results obtained in DMSO d_6 at 27 °C. 2D-COSY and Heteronuclear Multiple Quantum Coherence (HMQC) were run to obtain ^{1}H–^{1}H correlation and ^{1}H–^{13}C one-bond correlation to assist in the proton and carbon assignments. Finally, 2D-Heteronuclear Multiple Bond Correlation (HMBC) was run to obtain ^{1}H–^{13}C multi-bond correlations (two to three bonds) which provided the information needed to unequivocally assign most of the proton and carbon resonances. Furthermore, covalent bonding information between the functional groups could be deduced from the multi-bond ^{1}H–^{13}C correlations, which unambiguously confirmed the desired structure. The proton, carbon and fluorine assignments are given in Table 2 and details are provided in the ESI.

Table 2
^{1}H- ^{13}C- and ^{19}F NMR chemical shifts for compound **1**.



	Chemical Shifts (ppm)			
Atom	^{1}H	^{13}C	^{1}H (NH)	^{19}F
1				
2	3.02 4.42	55.8		
3	4.43	70.5		
4	1.84	25.8		
5/8	1.42 1.32 0.90	24.7 19.7		
6/7	2.74 2.62 2.54	47.4 46.4		
9				
10		155.1		
11				
12			3.54	
13		55.0		
14/15	1.60 1.56	30.3 29.8		
16		149.5		
17	7.56	123.6		
18		139.3		
19	7.45 dt	124.8		
20	7.39 t	129.1		
21	7.35	124.4		
22		137.4		
23/27	7.64 dd	129.1		
24/26	7.30 t	116.2		
25		162.3		
28				−115.8

Conclusion

A robust, reproducible 4-step convergent synthesis of the brain penetrant GCS inhibitor GZ667161 is presented that overcomes issues experienced with the previously reported method and provides the desired material in less steps and higher overall yield. The keys to the modified synthetic sequence were to move the Suzuki reaction to the beginning of the reaction sequence and incorporating the troublesome quinuclidine moiety in the last step. We have subsequently used this sequence to produce multi-gram quantities of GZ667161. The availability of this improved, efficient synthesis should facilitate the use of this important pharmacological tool molecule by researchers in the field of lysosomal storage disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Dr. Furong Sun and the team at the Mass Spectrometry Lab, School of Chemical Sciences, University of Illinois at Urbana-Champaign for providing the high resolution mass spectrometry data. The authors also thank Qualytics LLC, Warminster, PA, for providing detailed spectroscopic data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.152352.

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