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Modified Synthesis of the Peptidomimetic Natriuretic Peptide Receptor-C Antagonist M372049

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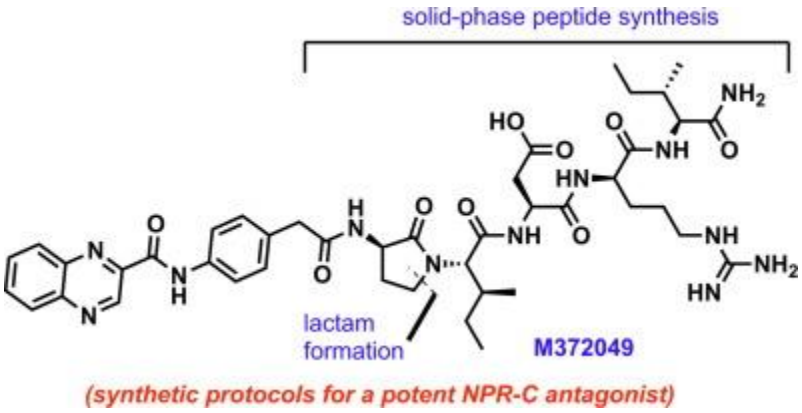
Highlights

- Details of a convenient, laboratory-scale synthesis of M372049 are disclosed.
- A chiral gamma lactam was prepared and characterized by X-ray crystallography.
- Solid-phase peptide synthesis was utilized without special equipment.

Abstract

The Natriuretic Peptide Receptors (NPRs) regulate vascular sodium levels and have been of significant interest for the potential treatment of hypertension and related cardiovascular complications. The peptidomimetic antagonist M372049 is a valuable probe for the study of NPR-C signaling, unfortunately it is presently not commercially available. Described is a detailed protocol for its synthesis that does not require specialized apparatus and builds upon a prior patent from Veale and colleagues. Key steps include a base-mediated lactam formation and a solid-supported peptide synthetic sequence. An X-ray crystal structure of a key lactam intermediate was obtained to confirm the structure and relative stereochemistry of the compound.

Graphical abstract



Abbreviations

Boc *tert*-butoxycarbonyl
DCE 1,2-dichloroethane
DCM dichloromethane
DMF *N,N*-dimethylformamide
EDC 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDT 1,2-ethanedithiol
Fmoc fluorenylmethyloxycarbonyl
HATU hexafluorophosphate azabenzotriazole tetramethyl uronium
HOBt 1-hydroxybenzotriazole
LC-MS liquid chromatography-mass spectrometry
PDA photodiode array
SPPS solid phase peptide synthesis
TFA trifluoroacetic acid

Keywords

Natriuretic peptide receptor-C, NPR-C antagonist, M372049, Peptidomimetic, Peptide synthesis, Lactam

Introduction

Natriuretic peptides (NPs) are hormones secreted in the cardiac atria in response to cardiovascular stress that bind and activate natriuretic peptide receptors (NPRs), leading to the excretion of sodium, vasodilation, and a decrease in blood pressure, among several effects [1]. One subtype of this receptor, NPR-C, is highly expressed in atrial myocardium and acts in part as a clearance receptor to decrease circulating levels of NPs, though it also transduces signals to the G protein Gi and decreases cAMP production. Most recently, NPR-C has also been implicated in persistent hypertension after repair of congenital aortic coarctation [2]. Truncated NPs have been investigated and are potent agonists of NPRs [3], [4], but presumably their plasma instability, rapid clearance, and/or lack of bioavailability have thus far precluded practical use. A possible alternative approach to the treatment of hypertension and other related cardiovascular disorders would be to inhibit NPR-C and increase the concentration of circulating NPs. This approach was reported by investigators at AstraZeneca [5], and the peptidomimetic M372049 (**1**) is presently considered to be the best-in-class NPR-C antagonist. However, its lack of commercial availability has precluded its use as a tool compound to support *in vivo* cardiovascular studies. Herein is described a detailed protocol for the preparation of this important compound [6], using the patent including M372049 from Veale and colleagues as a starting point [7].

We considered several syntheses of M372049, but the one which we ultimately pursued deviates only slightly from the patent, with the retrosynthesis outlined in Fig. 1. The molecule can be assembled via Fmoc-based solid phase peptide synthesis (SPPS) [8], with the key building blocks **2** and **3** prepared separately to increase the convergency of the synthesis. Given the valuable nature of lactam **3**, we ultimately explored using lower amounts of this building block in the SPPS to increase the efficiency.

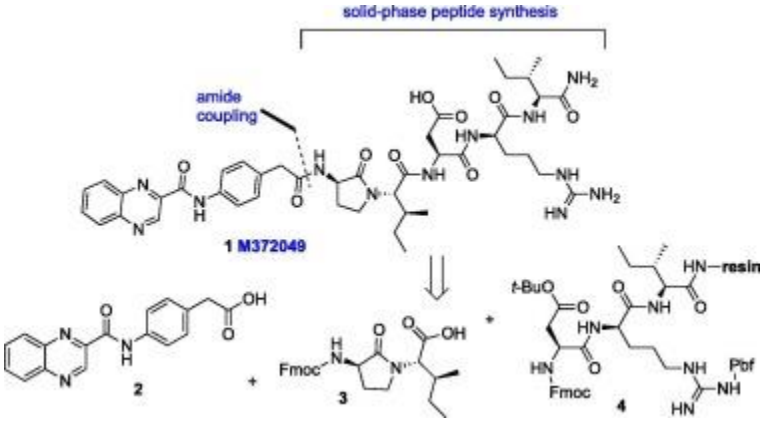


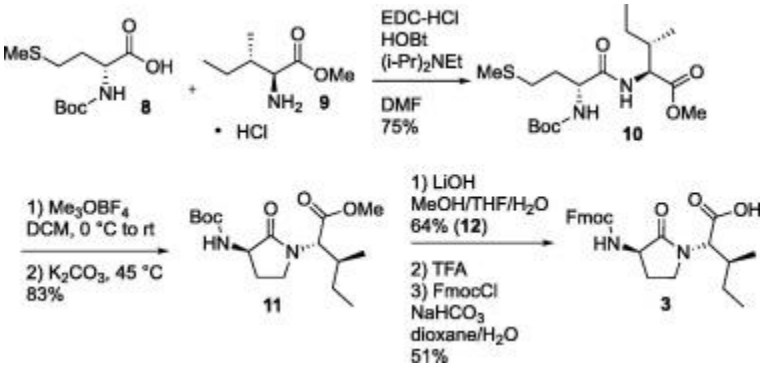
Fig. 1. Retrosynthesis of M372049 (**1**).

Results

We began by coupling quinoxaline-2-carboxylic acid **5** to aniline **6** to furnish amide **7** in high yield, followed by hydrolysis to give the desired acid **2** (Scheme 1). These were of acceptable purity to push forward without the need for further purification beyond the aqueous workup.

Scheme 1. Synthesis of western quinoxaline-amide-acid **2.**

The most challenging step in generating **1** was the efficient preparation of sufficient quantities of γ-lactam **3** for the SPPS portion of the synthesis (Scheme 2). This is an example of a "Freidinger lactam", which is a useful moiety for peptide conformational restriction [9], [10], [11]. The synthesis began with the amide coupling of *N*-Boc D-methionine (**8**) to isoleucine methyl ester (**9**), which generated dipeptide **10**. Formation of the lactam ring was facilitated by treatment of **10** with Meerwein's reagent to form a trialkylsulfonium leaving group, with the intermediate sufficiently stable for identification and monitoring by LC–MS. Addition of K_2CO_3 and heating for 12 h gave lactam **11.** IR analysis showed a shift in the amide carbonyl stretch from 1649 cm^{-1} in **10** to 1695 cm^{-1} in **11**, consistent with the conversion of a secondary amide to a γ-lactam, rather than a lactone. To confirm formation of the lactam and its stereochemical configurations, an X-ray crystal structure of **12** was obtained (Fig. 2, CCDC Deposition Number 1973363).



Scheme 2. Synthesis of γ-lactam **3**.

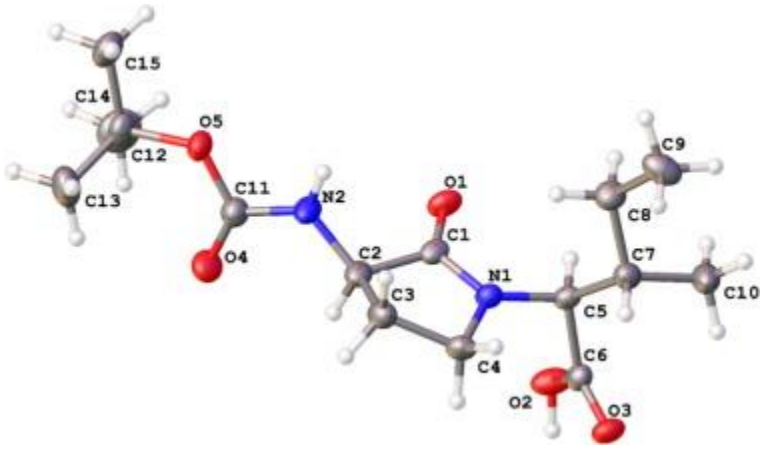
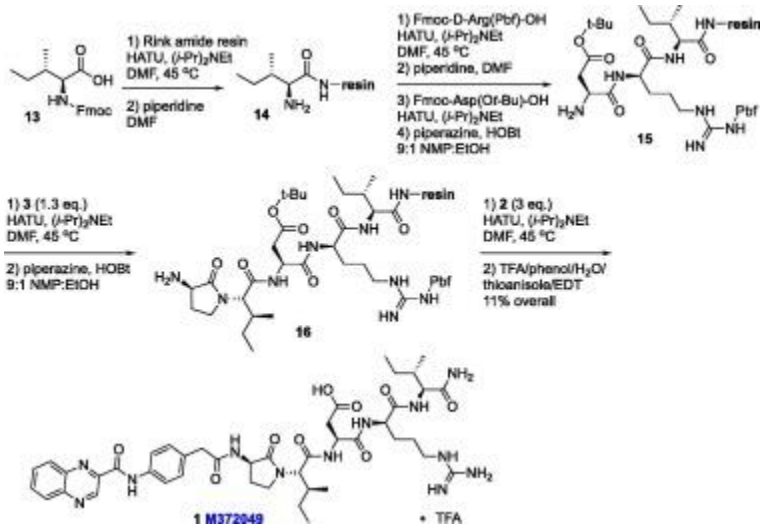


Fig. 2. X-ray structure of lactam **12**.

During the hydrolysis to give acid **12**, Veale and colleagues reported partial epimerization after the workup [7], which required a separate crystallization to obtain diastereomerically pure material. In our

protocol, dilution of the crude reaction mixture with H_2O and acidification with aqueous HCl led to precipitation of a white solid which was filtered off to cleanly give the desired product as a single diastereomer. Lastly, removal of the Boc group with TFA followed by treatment with FmocCl gave the Fmoc-protected γ-lactam to be used in the SPPS portion of the synthesis. All intermediates in the synthesis of **3** were able to be isolated in solid form, in high purity, without recourse to column chromatography.

SPPS was performed with a Rink amide resin according to common protocols (Scheme 3) [8], using Kaiser tests to qualitatively confirm the completion of all coupling and deprotection steps (see Supporting Info for details). Reactions were performed in glass vials without stirring, to avoid breaking down the resin to particles too small to filter, though a shaker could optionally be used. Initial attempts to couple standard peptides at room temperature were sluggish and did not go to completion without the use of large excesses of peptides. We found that using 3 equivalents of peptide and heating at 45 °C in minimal solvent for 3–12 h led to complete coupling. To prevent potential aspartamide formation during deprotection after the coupling of protected aspartic acid, the deprotection cocktail was switched from 20% piperidine in DMF to 5% piperazine in 0.1 M HOBt in 9:1 NMP:EtOH [12]. Given the valuable nature of lactam **3**, we explored lower substrate loadings for this step. We found that the use of 1.3 equivalents of **3** and heating at 45 °C gave a complete reaction within 12 h. The final coupling of quinoxaline-acid **2** was run under our standard peptide coupling conditions, given the ease at which gram quantities of this compound could be produced. After treatment of the resin with a TFA-based deprotection cocktail at room temperature for 4 h, 1.2 g of crude material was obtained (1.7 g theoretical yield). Resubjection of the resin to the deprotection cocktail at 60 °C for 12 h did not yield a further significant quantity of crude material (47 additional milligrams obtained). The crude final product **1** was purified using automated flash chromatography on a 30 g C18 column and eluted with a 0.1% TFA in acetonitrile–water gradient. Pooling of fractions which contained the desired product gave 215 mg of material with minor impurities which could not be separated via flash chromatography. Fortunately, these impurities could be removed by trituration with 9:1 ether:chloroform. The synthesis yielded 195 mg of desired product (11% overall yield) at >99% purity without the need for preparative HPLC purification. ^{19}F NMR with 1,4-difluorobenzene as internal standard confirmed that **1** was isolated as the mono-TFA salt.



Scheme 3. Assembly of M372049 via SPPS.

Conclusion

Alternatively, the three C-terminal residues of the peptidomimetic could be connected by solution phase, then joined to the preformed western half of M372049 containing the γ-lactam. Since the C-terminal tripeptide was synthesized rapidly on solid support without recourse to chromatography, and since the on-resin synthesis did not require stirring or special apparatus, it is highly scalable, and a fully solution phase synthesis would seem to offer no obvious advantages. In conclusion, an efficient, scalable protocol for the synthesis of the NPR-C antagonist M372049 is disclosed.

Author contributions

Planned the synthesis: J.P., C.D. Synthesized and characterized analogs: J.P. Obtained single crystals and determined X-ray structure: J.P., S.L. Wrote the manuscript and Supporting Information: C.D., J.P.

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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.

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Appendix A. Supplementary data

The following are the Supplementary data to this article: Download : **Download Acrobat PDF file (2MB)** Supplementary Data 1.

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