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Synthesis and Library Construction of Privileged Tetra-Substituted Δ^5-2-Oxopiperazine as β-Turn Structure Mimetics

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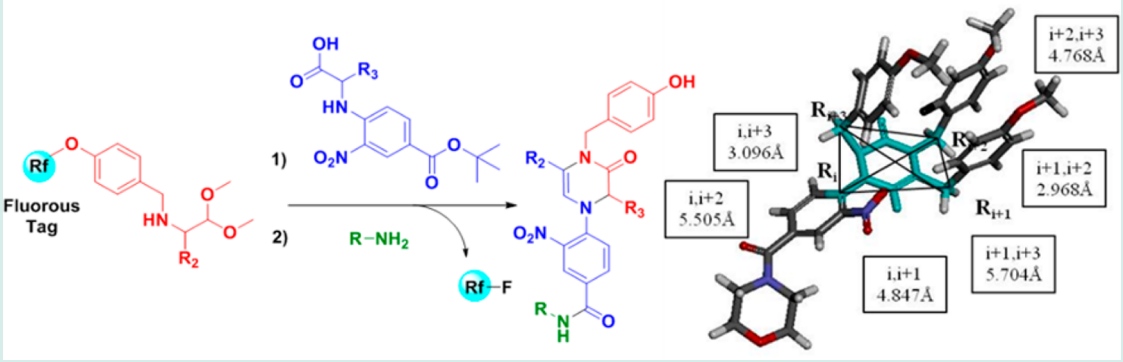
Supporting Information



ABSTRACT: In this study, we developed an efficient and practical procedure for the synthesis of tetra-substituted Δ^5-2-oxopiperazine that mimics the bioactive β-turn structural motif of proteins. This synthetic route is robust and modular enough to accommodate four different substituents to obtain a high level of molecular diversity without any deterioration in stereochemical enrichment of the natural and unnatural amino acids. Through the *in silico* studies, including a distance calculation of side chains and a conformational overlapping of our model compound with a native β-turn structure, we successfully demonstrated the conformational similarity of tetra-substituted Δ^5-2-oxopiperazine to the β-turn motif. For the library construction in a high-throughput manner, the fluorous tag technology was adopted with the use of a solution-phase parallel synthesis platform. A 140-membered pilot library of tetra-substituted Δ^5-2-oxopiperazines was achieved with an average purity of 90% without further purification.

KEYWORDS: *Δ^5-2-oxopiperazine, synthesis, library construction, mimetics*

INTRODUCTION

Since the completion of the Human Genome Project, the development of novel small-molecule modulators toward various gene products has been a key research field of interest. Their great potential for use in the elucidation of gene functions and the associated control of gene products, as well as being prospective new therapeutic agents, make them hugely valuable.[1] In particular, the newly emerging interdisciplinary research area of chemical biology has been focused on the identification of novel bioactive small-molecules that can specifically modulate biological phenomena, such as protein−protein interactions and the associated signaling pathways, in order to elucidate and gain control over their mechanism of action.[2] Historically, the majority of small-molecule modulators and potential therapeutic agents have been identified through biological screening using a library of natural products.[3] However, the development of high-throughput screening technology has led to a huge demand for collections of novel drug-like and natural product-like small molecules with maximized molecular diversity to the field of chemical biology and the pharmaceutical industry.[4] To address this unmet need, the synthetic chemistry community has embraced the diversity-oriented synthesis (DOS) strategy, which aims to provide an efficient generation of complex and diverse compound libraries that contain a large number of structurally diverse molecular frameworks.[5] Our group has been focused on the development of divergent synthetic routes for the systematic construction of libraries of drug-like polyheterocycles embedded with privileged substructures, including benzopyran,[6] benzodiazepine,[7] pyrazole,[8] pyrazolopyridine,[9] and tetrahydroindazolone.[10] We named this approach a privileged-substructure-based diversity-oriented synthesis (pDOS).[11] As a continuation of our previous efforts in pDOS and molecular diversity, we aimed to develop a new robust route for the synthesis of tetra-substituted Δ^5-2-oxopiperazine.

Oxopiperazine is an important pharmacophore that mimics the β-turn motif, which is one of the three main secondary structures found in proteins and peptides (α-helix, β-turn, or β-strand).[12] In fact, a number of biologically interesting peptides, specifically observed in protein−protein interactions, contain

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the β-turn motif in important recognition segments.[13,14] Therefore, oxopiperazine can serve as an attractive molecular framework that mimics the key interaction residues and uniquely displays the recognition motif in three-dimensional space.[15] As shown in Figure 1, Δ^5-2-oxopiperazine moieties are

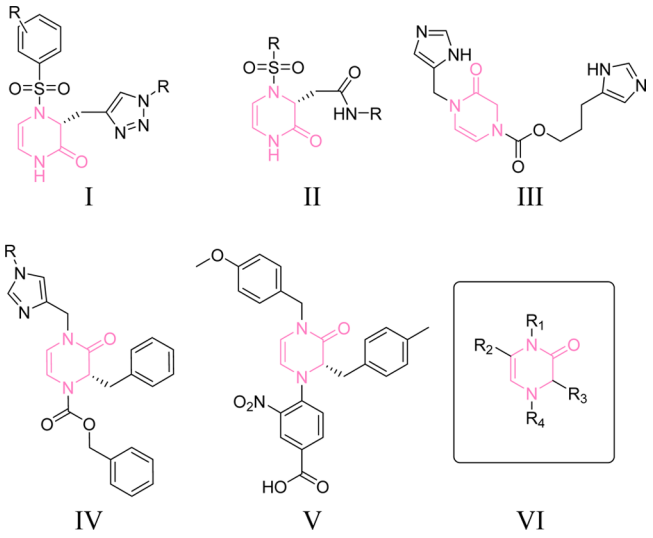


Figure 1. Bioactive small molecules with a Δ^5-2-oxopiperazine moiety: (I, II) Bradykinin receptor antagonists, (III) GGTI-2410, (IV) GGTI-2421: R = Boc, GGTI-2422: R = H, (V) Chondrogenamine (chondrogenesis inducer), (VI) our tetra-substituted Δ^5-2-oxopiperazine molecules.

frequently observed in bioactive small molecules. For example, Bradykinin receptor antagonists (I, II) mimic the peptide–receptor interaction as anti-inflammatory drugs.[16] Geranylgeranyltransferase I (GGTase-I) inhibitor (III) and farnesyltransferase (FTase) inhibitor (IV) mimic the hotspot of peptide–enzyme interaction. In addition, there are numerous other bioactive compounds embedded with Δ^5-2-oxopiperazine.[17]

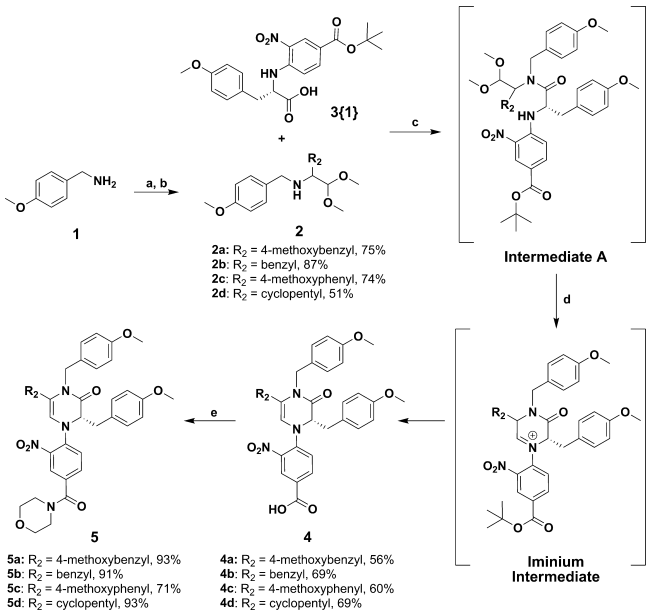
The principal synthetic procedure for Δ^5-2-oxopiperazine is through formation of iminium ions from aldehydes or their synthons with amines via intramolecular cyclization and subsequent olefin migration or reduction.[18,19] However, the previously reported methods have a number of limitations that prevent their application to the synthesis of diverse drug-like compounds, including difficulty in controlling the stereochemistry due to the requirement of strongly acidic conditions. To tackle these problems, we previously developed a novel synthetic method for Δ^5-2-oxopiperazine derivatives using mild acidic conditions. High-throughput construction of trisubstituted Δ^5-2-oxopiperazines such as V as γ-turn mimetics was achieved using a solid-phase parallel synthesis platform (Figure 1).[19,20] The resulting trisubstituted Δ^5-2-oxopiperazine library was subjected to various cell-based high-throughput assays, such as cell proliferation assay, reporter gene assay, image-based phenotypic assay, and stem-cell differentiation assay. Through these screenings, we identified chondrogenamine [Figure 1(V)], a novel small-molecule modulator containing Δ^5-2-oxopiperazine that induces chondrogenic differentiation of human bone-marrow-derived mesenchymal stem cells.[20] This result emphasizes the importance of the development of new synthetic pathways for drug-like small molecules that mimic the structural motifs of gene products. As a continuation of our efforts in maximizing molecular diversity, we aimed to synthesize tetra-substituted Δ^5-2-oxopiperazine [Figure 1(VI)], which can mimic the β-turn structure, the most frequently observed turn structure in protein–protein interactions. The major difference between β-turns and γ-turns is the number of amino acid side chains on the molecular frame: Compared to γ-turns that consist of three amino acid side chains, β-turns contain an additional side chain.[21] Therefore, the introduction of additional substituents on the Δ^5-2-oxopiperazine structural motif would be essential for mimicking β-turn structure along with additional diversification of its core skeleton.

RESULTS AND DISCUSSION

Herein, we report the development of an efficient synthetic pathway for tetra-substituted Δ^5-2-oxopiperazine as a β-turn mimetic, and the subsequent high-throughput construction of a drug-like small molecule library based on this molecular framework. In our previous report, we successfully demonstrated the practical solid-phase parallel synthesis of trisubstituted Δ^5-2-oxopiperazine via sequential transformations in a single operation: acidolytic cleavage of masked aldehydes from solid supports, subsequent cyclic iminium formation, and double bond rearrangement.[19,20] This original solid-phase synthetic procedure allowed the construction of a pilot library containing a trisubstituted Δ^5-2-oxopiperazine core skeleton, which mimics the dipeptidic γ-turn structure. For the expansion of molecular diversity, we then aimed to achieve a robust synthesis of tetra-substituted Δ^5-2-oxopiperazine, which can mimic a tripeptidic β-turn structure instead of a γ-turn. However, it is inherently difficult to introduce additional substituents on Δ^5-2-oxopiperazine moieties using our existing methods involving the solid-phase synthetic platform.

As illustrated in Scheme 1, the original synthetic procedure for the Δ^5-2-oxopiperazine moiety was modified to accom-

Scheme 1. Synthetic Procedure for Tetra-Substituted Δ^5-2-Oxopiperazines with a Diversification Point[a]



[a]Reagents and conditions: (a) 2,2-dimethoxyacetaldehyde, Na_2SO_4, DCM, r.t, 3 h. (b) R_2-MgX, THF, r.t, 1 h. (c) EDC, DCM, r.t. (d) Neat formic acid, r.t, 1 h. (e) Morpholine, EDC, DMAP, DCM, r.t.

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modate various R_2 substituents, such as 4-methoxybenzyl (**a**), benzyl (**b**), 4-methoxyphenyl (**c**), and cyclopentyl (**d**), via the reductive alkylation of imine intermediates with Grignard reagents as R_2 substituents. The imine intermediate was formed with 4-methoxybenzyl amine (**1**) and 2,2-dimethoxyaldehyde in anhydrous dichloromethane (DCM) solution at room temperature and the subsequent reductive alkylation with various Grignard reagents allowed the formation of secondary amines **2a−2d** in moderate to excellent yields. To confirm the robustness of this synthetic route, we pursued the synthesis of representative compounds **5a−5d** with fixed substituents, such that R_1 and R_3 were 4-methoxybenzyl and R_4 was a morpholinocarbonyl 3-nitrophenyl moiety. Compound **3{1}** was formed via nucleophilic aromatic substitution of free amino acids with electron-deficient *tert*-butyl-4-fluoro-3-nitrobenzoate and coupled with compound **2** via an amide bond using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) to form intermediate **A**. However, unexpectedly, significant amounts of unidentified byproducts were observed under the standard conditions for amide bond formation. After extensive screening with various coupling reagents, temperatures, and bases, intermediate **A** was obtained with high efficiency by using the optimized conditions of EDC in the absence of a base and a catalyst in dichloromethane (DCM) at room temperature for a prolonged reaction time (data not shown). Following this, intermediate **A** was subjected to neat formic acid without purification to obtain compound **4** through a series of transformations: acidolysis of acetal to yield aldehyde, iminium ion formation by reaction of resulting aldehyde with a secondary amine, acid-catalyzed rearrangement of an iminium ion to enamine to yield the Δ^5-2-oxopiperazine core skeleton, and *in situ* removal of the *tert*-butyl protecting group. These four sequential chemical transformations were carried out in one pot with high efficiency. It is worth mentioning that the electron-withdrawing nitro group on the aryl moiety at the R_4 position plays an important role in the efficient rearrangement of the iminium ion to enamine. In addition, this nitro moiety can serve as a potential functional handle for further diversification after its reduction to amine. Using this solution-phase synthetic route, we successfully prepared representative tetra-substituted Δ^5-2-oxopiperazines **5a−5d** in good to excellent yields, which confirms the robustness of this route. In this pilot study, we focused on the diversification of carboxylic acid group in **4a−4d** through amide coupling with amines, such as morpholine, to maximize the molecular diversity.

We also carried out an investigation into whether the representative tetra-substituted Δ^5-2-oxopiperazine **5a** was conformationally similar to the β-turn structure. As shown in Figure 2A, the β-turn structure consists of four amino acid residues, designated as i, $i + 1$, $i + 2$, and $i + 3$. One of the important features of the β-turn structure is that the distance between the $C_{\alpha i}$ and the $C_{\alpha i+3}$ atoms is less than 7 Å.[21] The energy-minimized structure of compound **5a** was virtually constructed, and the distances between each of substituents of compound **5a** were calculated. As shown in Figure 2B, the largest distance gap between substituents was found to be 5.704 Å, which is consistent with the important characteristic of the β-turn structure. In addition, the orientations of individual substituents in **5a** were well aligned with those of the side chains in the β-turn structure (Figure 2C). For further confirmation, the energy-minimized 3D structure of tetra-substituted Δ^5-2-oxopiperazine **5a** was overlapped with the β-turn crystal structure of gonadotropin-releasing hormone (GnRH), one of the peptides containing a β-turn structural motif in its biologically active conformation (PDB ID of GnRH: 1YY1).[22] As shown in Figure 2D, **5a** overlapped well with the β-turn backbone structure of GnRH, which consists of tyrosine-glycine-leucine (in sky blue), with excellent conformational similarity. As a result, it was successfully demonstrated that the introduction of new substituents at the R_2 position transformed the original trisubstituted Δ^5-2-oxopiperazines as γ-turn mimetics to tetra-substituted Δ^5-2-oxopiperazines as β-turn mimetics.

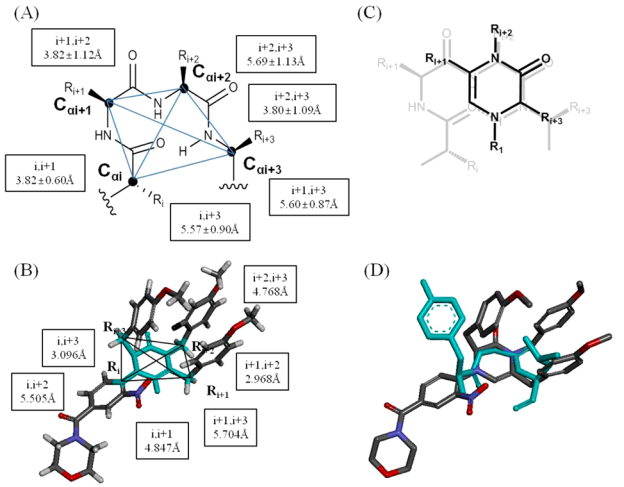

Figure 2. (A) Schematic diagram of a secondary structure of β-turn motif. (B) Distances between substituents in the energy-minimized conformer of tetra-substituted Δ^5-2-oxopiperazine **5a**. (C) β-turn mimicry by tetra-substituent Δ^5-2-oxopiperazine **5a**. (D) Overlay of compound **5a** with β-turn backbone motif (Tyr-Gly-Leu in sky blue) in gonadotropin-releasing hormone (GnRH, PDB: 1YY1) [PreADMET, V_{conf} Interface, Discovery studio 3.0].

For the practical construction of a tetra-substituted Δ^5-2-oxopiperazine compound library, a high-throughput synthesis platform was sought. As previously mentioned, the solid-phase parallel synthesis route cannot be applied for the synthesis of tetra-substituted Δ^5-2-oxopiperazines due to intrinsic difficulty in introducing an additional substituent through reductive alkylation of secondary amine with aldehyde. Therefore, a fluorous tag-based high-throughput strategy using solution-phase parallel synthesis was employed. A 4-hydroxybenzyl moiety was selected as a biased R_1 substituent not only because it mimics the tyrosine side chain in the β-turn structure but also because a perfluorosilane tag can be easily attached through a phenolic hydroxyl group. As shown in Scheme 2, library synthesis was initiated by the preparation of fluorous tagged secondary amines **7a−7d** with various R_2 substituents. After the imine formation from 4-hydroxybenzylamine **6** with 2,2-dimethoxyacetaldehyde in DCM solution, various Grignard reagents were added to the reaction mixture for the introduction of four different R_2 substituents, including 4-methoxybenzyl (**a**), benzyl (**b**), 4-methoxyphenyl (**c**), and cyclopentyl (**d**) moieties, via reductive alkylation. The resulting secondary amines containing a phenolic moiety were selectively protected with the fluorous tag under the anhydrous DCM solution in the presence of 2,6-lutidine after the acid-catalyzed activation of diisopropyl-(1*H*,1*H*,2*H*,2*H*-perfluorododecyl)-silane in DCM.

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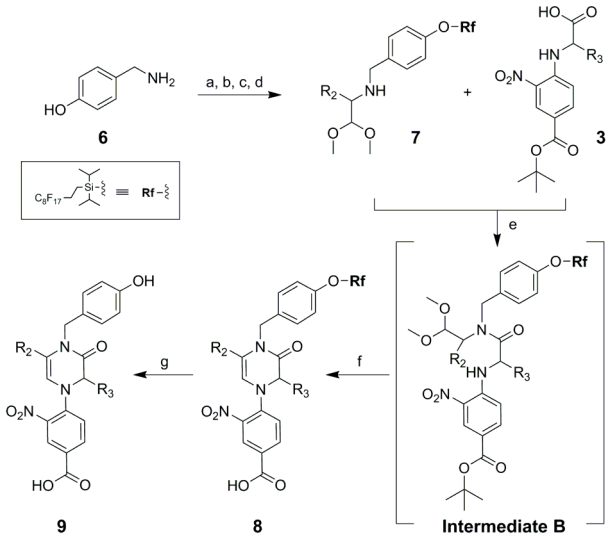
Scheme 2. General Synthetic Scheme for Tetra-Substituted Δ^5-2-Oxopiperazines Using Fluorous Tag-Based Solution-Phase Parallel Synthesis, and the Purity of Compounds 9{a−d,1−5}[a,b]



R2 \ R3	1	2	3	4	5
a	9{a,1}	9{a,2}	9{a,3}	9{a,4}	9{a,5}
b	9{b,1}	9{b,2}	9{b,3}	9{b,4}	9{b,5}
c	9{c,1}	9{c,2}	9{c,3}	9{c,4}	9{c,5}
d	9{d,1}	9{d,2}	9{d,3}	9{d,4}	9{d,5}

Purity: >90%, 80-90%, 70-80%, <70%

[a]Reagents and conditions: (a) 2,2-dimethoxyacetaldehyde, Na_2SO_4, DCM, r.t, 3 h. (b) R_2-MgX, THF, r.t, 1 h. (c) Diisopropyl-(1*H*,1*H*,2*H*,2*H*-perfluorododecyl)silane, p-TfOH, DCM, 0 °C to r.t, 15 h. (d) 2,6-Lutidine, DCM, r.t, 3 h. (e) EDC, DCE, 50 °C. (f) Formic acid, DCM, r.t, 1 h. (g) HF/pyridine/THF (5:5:90), r.t, 2 h. [b]Purities were obtained using PDA-based LC/MS analysis of crude final compounds after removal of fluorous tag

Then, secondary amine intermediates containing a fluorous tag (**7a**−**7d**) were coupled with compound **3** using EDC-based amide coupling in 1,2-dichloroethane (DCE). Five different benzoate-containing intermediates **3{1}**−**3{5}** were prepared from the diversification of *tert*-butyl-4-fluoro-3-nitrobenzoate with natural or unnatural amino acids, such as L-methoxyphenylalanine, L-valine, D-valine, L-4-fluorophenylalanine and L-glutamate methyl ester, respectively. Inconsistent with our above-mentioned solution-phase study, we observed the significant deterioration of reaction yields in the amide coupling of **7a**−**7d** with **3**, which is probably due to the low reactivities of fluorous tagged secondary amines **7a**−**7d**. Therefore, the amide coupling reaction was pushed to completion by mild heating to 50 °C for 14 h without a significant decrease in yields. After the completion of the reaction, the desired intermediate **B** was easily purified through fluorous solid-phase extraction (F-SPE) without silica-gel flash column chromatography. Intermediate **B** was then subjected to the key transformation for the synthesis of the Δ^5-2-oxopiperazine core skeleton. Unlike in our earlier solution-phase study, intermediate **B** was treated with 90% formic acid in DCM rather than neat formic acid because of the poor solubility of intermediate **B** in the neat formic acid. Under this acidic environment, intermediate **B** can be successfully converted to the desired tetra-substituted Δ^5-2-oxopiperazine **8** through a series of chemical transformations: acidolytic formation of an aldehyde moiety, iminium ion formation, rearrangement of the iminium ion to Δ^5-2-oxopiperazine, and the *in situ* removal of the *tert*-butyl protecting group. After removal of the fluorous tag by treatment with hydrofluoric acid (HF)/pyridine and subsequent quenching with ethoxytrimethylsilane (TMSOEt), purities of 20 final compounds **9{a−d,1−5}** were assessed using a PDA-equipped LC/MS without any further purification (average purities: 95%). Five representative compounds, **9{a,1}**, **9{a,2}**, **9{a,3}**, **9{c,5}**, and **9{d,1}**, were fully characterized using ^{1}H and ^{13}C NMR spectroscopy along with LC/MS analysis (see Table 1 and the Supporting Information).

Table 1. Purity and Mass Conformation of Representative Compounds 9{a−d,1−5}

	R_2	R_3	Yield [a] (%)	Purity [b] (%)	MS[M+H]$^+$ calcd	MS[M+H]$^+$ found
9{a,1}			50	93	610.21	610.08
9{a,2}			49	99	532.20	532.05
9{a,3}			43	99	532.20	532.11
9{c,5}			42	90	562.17	562.13
9{d,1}			47	93	558.22	558.07

[a]Overall percentage yields were calculated after removal of fluorous tag and F-SPE. [b]Purities were obtained using PDA-based LC/MS analysis of crude final compounds after removal of fluorous tag.

The resulting fluorous tagged tetra-substituted Δ^5-2-oxopiperazines **8** containing a free carboxylic acid were further diversified with various amines, including diethylamine, 1-methylpiperazine, morpholine, *N*-ethyl-2-methoxyethylamine, 2-methylpropylamine, and (*S*)-(tetrahydrofuran-2-yl)-methanamine, as R_5 substituents through HATU-based amide coupling in *N*,*N*-dimethylformamide (DMF) containing *N*,*N*-diisopropylethylamine (DIPEA). As shown in Scheme 3, intermediate **C** with the fluorous tag can be easily purified by simple solid-phase extraction, and the final compounds **10{R_2,R_3,R_5}** were obtained after removal of fluorous tag using HF/pyridine without any further purification. Representative tetra-substituted Δ^5-2-oxopiperazines, **10{a,2,3}**, **10{b,3,6}**, **10{c,3,1}**, **10{c,5,6}**, and **10{d,1,3}**, were fully characterized using ^{1}H and ^{13}C NMR spectroscopy along with LC/MS analysis (see Table 2 and the Supporting Information). Using this fluorous tag-based high-throughput synthesis platform, we efficiently constructed a 120-member drug-like library of tetra-substituted Δ^5-2-oxopiperazines on a scale of approximately 10 mg each. The purities of all library members were measured using LC/MS equipped with a PDA detector without further purification, and their average purity was found to be 89% after five chemical transformations.

To visualize the molecular diversity and distinctive characteristics of the β-turn mimicking tetra-substituted Δ^5-2-oxopiperazine library, we performed *in silico* analysis of this 140-member library along with the direct comparison with γ-turn mimicking trisubstituted Δ^5-2-oxopiperazines. Both tri- and tetra-sub-

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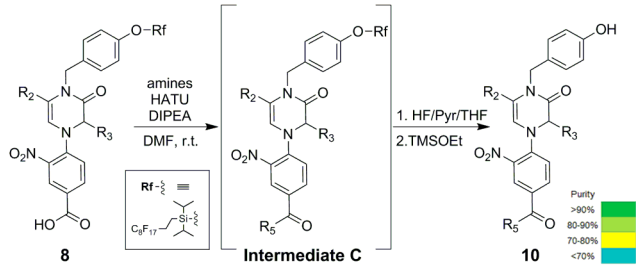
Scheme 3. High Throughput Construction of 120-Member Library of Tetra-Substituted Δ^5-2-Oxopiperazine 10{a−d,1−5,1−6} through Amide Coupling of Intermediate 8 Containing Benzoic Acid with Various Amines (R_5) and the Purity of All Final Compounds[a]



R_2	R_5	R_3 1	2	3	4	5
a	1	10{a,1,1}	10{a,2,1}	10{a,3,1}	10{a,4,1}	10{a,5,1}
	2	10{a,1,2}	10{a,2,2}	10{a,3,2}	10{a,4,2}	10{a,5,2}
	3	10{a,1,3}	10{a,2,3}	10{a,3,3}	10{a,4,3}	10{a,5,3}
	4	10{a,1,4}	10{a,2,4}	10{a,3,4}	10{a,4,4}	10{a,5,4}
	5	10{a,1,5}	10{a,2,5}	10{a,3,5}	10{a,4,5}	10{a,5,5}
	6	10{a,1,6}	10{a,2,6}	10{a,3,6}	10{a,4,6}	10{a,5,6}
b	1	10{b,1,1}	10{b,2,1}	10{b,3,1}	10{b,4,1}	10{b,5,1}
	2	10{b,1,2}	10{b,2,2}	10{b,3,2}	10{b,4,2}	10{b,5,2}
	3	10{b,1,3}	10{b,2,3}	10{b,3,3}	10{b,4,3}	10{b,5,3}
	4	10{b,1,4}	10{b,2,4}	10{b,3,4}	10{b,4,4}	10{b,5,4}
	5	10{b,1,5}	10{b,2,5}	10{b,3,5}	10{b,4,5}	10{b,5,5}
	6	10{b,1,6}	10{b,2,6}	10{b,3,6}	10{b,4,6}	10{b,5,6}
c	1	10{c,1,1}	10{c,2,1}	10{c,3,1}	10{c,4,1}	10{c,5,1}
	2	10{c,1,2}	10{c,2,2}	10{c,3,2}	10{c,4,2}	10{c,5,2}
	3	10{c,1,3}	10{c,2,3}	10{c,3,3}	10{c,4,3}	10{c,5,3}
	4	10{c,1,4}	10{c,2,4}	10{c,3,4}	10{c,4,4}	10{c,5,4}
	5	10{c,1,5}	10{c,2,5}	10{c,3,5}	10{c,4,5}	10{c,5,5}
	6	10{c,1,6}	10{c,2,6}	10{c,3,6}	10{c,4,6}	10{c,5,6}
d	1	10{d,1,1}	10{d,2,1}	10{d,3,1}	10{d,4,1}	10{d,5,1}
	2	10{d,1,2}	10{d,2,2}	10{d,3,2}	10{d,4,2}	10{d,5,2}
	3	10{d,1,3}	10{d,2,3}	10{d,3,3}	10{d,4,3}	10{d,5,3}
	4	10{d,1,4}	10{d,2,4}	10{d,3,4}	10{d,4,4}	10{d,5,4}
	5	10{d,1,5}	10{d,2,5}	10{d,3,5}	10{d,4,5}	10{d,5,5}
	6	10{d,1,6}	10{d,2,6}	10{d,3,6}	10{d,4,6}	10{d,5,6}

[a]Purities were obtained using PDA-based LC/MS analysis of crude final compounds after removal of the fluorous tag.

stituted Δ^5-2-oxopiperazine libraries were subjected to computational calculation with 15 major molecular descriptors by using PreADMET 2.0 [BMDRC, Seoul, Korea], such as hydrophobic surface area, molecular weight, ALogP, topological polar surface area, van der Waals (VDW) volume, electronegativity, number of hydrogen bond acceptors and donors, etc. Three principal components (Prin1, Prin2, and Prin3) represent 99.7% of the total variance in molecular descriptors. Prin1 factor, which explains 93.8% of the total variance, is mainly constituted by hydrophobic surface area (SA)_MPEOE, 2D VDW surface, and molecular weight. Prin2 factor, which explains 4.7% of the total variance, is influenced by molecular weight, 2D_VDW_volume, and topological polar surface area. Prin3 factor, accounting for 1.2% of the total variance, includes topological polar surface area, hydrophobic SA_MPEOE, and 2D_ polar van der Waals surface area (VSA). As shown in Figure 3A, the resulting principle component analysis (PCA) clearly visualized that the library members of tetra-substituted Δ^5-2-oxopiperazine (yellow and green dots) are widely distributed in the 3D chemical space distinct from the representative collection of trisubstituted Δ^5-2-oxopiperazine (red dots). On the basis of this *in silico* analysis, we are confident that the tetra-substituted Δ^5-2-oxopiperazine scaffold can significantly expand the molecular diversity of drug-like oxopiperazine structures. Therefore, both scaffolds should be extremely valuable in a complementary manner because they expand the molecular diversity of privileged oxopiperazines in their own ways and populated their molecular diversity in the different region of 3D chemical space. It is also worth mentioning that tetra- and trisubstituted Δ^5-2-oxopiperazine scaffolds are mimicking the complementary β-turn and γ-turn motif, respectively, whose importance cannot be overstated in the recognition events in biopolymers. In addition, we would like to emphasize that the introduction of various R_5 substituents successfully expanded the molecular diversity of the resulting tetra-substituted Δ^5-2-oxopiperazine library, which was visualized in different colors (see Figure 3b).

CONCLUSION

In this study, we designed and synthesized tetra-substituted Δ^5-2-oxopiperazines that can mimic the β-turn structure, one of the key secondary structural motifs involved in protein−protein interactions. Through distance calculations and the alignment of our model compound **5a** with side chains of a biologically active β-turn structure, we confirmed that the tetra-substituted Δ^5-2-oxopiperazine could acquire conformational similarity to the β-turn structure through the introduction of new substituents on the skeleton of a trisubstituted Δ^5-2-oxopiperazine, which mimics the γ-turn structure. Distinct from our original synthesis of trisubstituted Δ^5-2-oxopiperazine, a fluorous tag-based solution-phase strategy was adopted as a high-throughput parallel synthesis platform to ensure the efficient synthesis and purification of desired compounds with a simple F-SPE-based filtration. For the library construction, the R_1 substituent was fixed with 4-hydroxybenzyl moiety, identical to the side chain of tyrosine, because the common phenolic group can serve as a functional handle to attach the perfluorosilane tag for fluorous solid-phase extraction. In addition, the unsaturated oxopiperazine core skeleton was decorated with R_2 and R_3 substituents using various Grignard reagents and chiral amino acids, respectively. The mild and robust procedure of our route allowed the synthesis of final compounds as single enantiomers through maintaining the enantiopurity from chiral amino acids. Therefore, molecular diversity of the tetra-substituted Δ^5-2-oxopiperazine library can be further expanded, not only with building block diversity but also with stereochemical diversity. In this pilot library synthesis, 3-nitrobenzoic acid was used as the sole R_4 substituent, owing

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Table 2. Purity and Mass Conformation of Representative Compounds 10{a−d,1−5,1−6}

	R_2	R_3	R_5	Yield[a] (%)	Purity[b] (%)	MS[M+H]$^+$ calcd	MS[M+H]$^+$ found
10{a,2,3}				80	95	601.26	601.22
10{b,3,6}				83	76	585.26	585.21
10{c,3,1}				86	95	573.26	573.25
10{c,5,6}				79	99	645.25	645.26
10{d,1,3}				85	97	627.27	627.19

[a]Overall percentage yields were calculated after removal of the fluorous tag and F-SPE. [b]Purities were obtained using PDA-based LC/MS analysis of crude final compounds after removal of the fluorous tag.

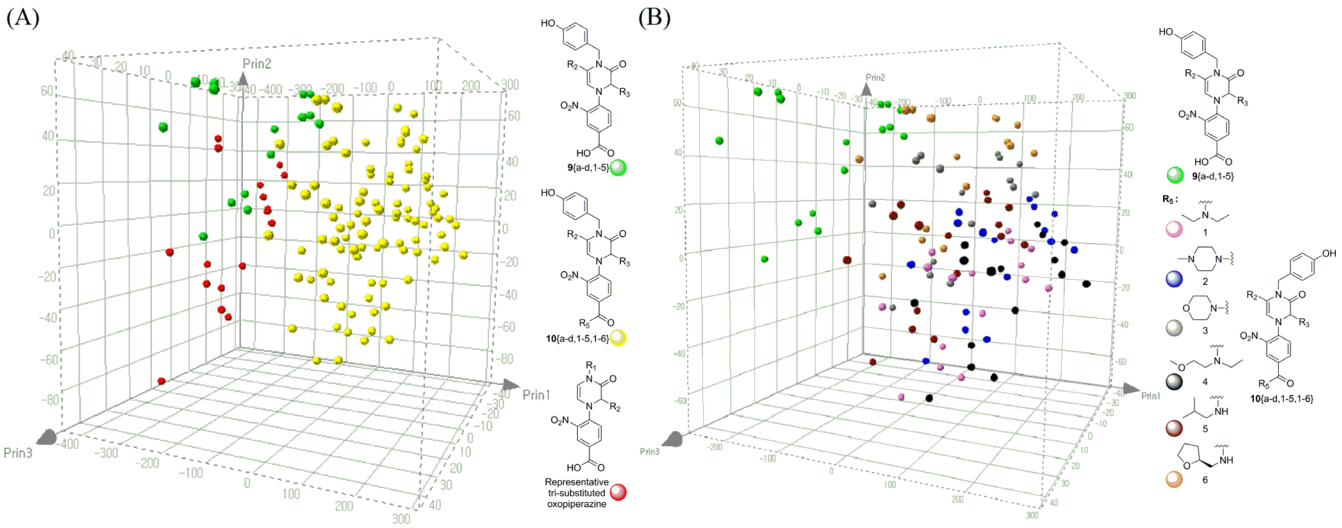

Figure 3. (A) Principle component analysis (PCA) of the 140-member compound collection and representative trisubstituted Δ^5-2-oxopiperazines.[20] (B) 3-D visualization of chemical space of tetra-substituted Δ^5-2-oxopiperazine library differentiated by R_5 substituents. [PreADMET, V_{conf} Interface, SAS 9.2, Spotfire Decision site].

to its electron withdrawing effect that accelerated the initial nucleophilic aromatic substitution and the rearrangement of the iminium to enamine for the formation of the unsaturated oxopiperazine core skeletons. The molecular diversity of library members can be expanded via the selective reduction of this nitro group and subsequent amine modifications. Instead, in this study, we successfully demonstrated the introduction of R_5 substituents through amide coupling of benzoic acids. In summary, we constructed a 140-membered tetra-substituted Δ^5-2-oxopiperazine pilot library as β-turn mimetics, without further purification, with an average purity of 90% using a robust and efficient synthetic method. The biological evaluation of the resulting tetra-substituted Δ^5-2-oxopiperazines will be reported in due course.

EXPERIMENTAL PROCEDURES

General Information. All commercially available reagents and solvents were used without further purification unless noted otherwise. All solvents were purchased from commercial suppliers. Diisopropyl-(1*H*,1*H*,2*H*,2*H*-perfluorododecyl)silane was purchased from Fluorous Technologies Inc. (Pittsburgh, PA, USA). Analytical thin-layer chromatography (TLC) was performed using 0.25 mm silica-gel-coated Kiselgel 60 F_{254} plates, and the components were visualized by observation under UV light (254 and 365 nm) or by treating the plates with ninhydrin followed by thermal visualization. ^{1}H, ^{13}C NMR spectra were obtained using Bruker DRX-300 [Bruker Biospin, Germany], Varian DD2MR400, and Varian Inova-500 [Varian Assoc., USA]. Chemical shifts were reported in parts per million (ppm) from tetramethylsilane (TMS) as an internal standard or the residual solvent peak. Multiplicities were indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), dt (doublet of triplet), td (triplet of doublets), and br s (broad singlet). Coupling constants were reported in hertz. The purities of all library members were determined using an LC/MS system equipped with a reverse-phase column (C-18, 50 × 2.1 mm, 5 μm) and a photodiode array (PDA) detector using electronic spray ionization (ESI).

General Synthetic Procedure for Compound 2. To a solution of 4-methoxybenzyl amine in dichloromethane (DCM), 2,2-dimethoxyacetaldehyde (2.0 equiv) and anhydrous Na_2SO_4(s) (30 equiv) were added, and the reaction mixture was stirred at room temperature for 3 h. After the completion of imine formation monitored by TLC, the reaction mixture was filtered and condensed under reduced pressure. The residue was dissolved in anhydrous tetrahydrofuran (THF), and Grignard reagent (3.0 equiv) was added to this solution at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature for 1 h. After the completion of reaction monitored by TLC, the reaction mixture was quenched by the

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addition of methanol and concentrated *in vacuo*. The residue was redissolved in DCM, and the organic layer was washed with saturated NH_4Cl(aq) and brine, dried over anhydrous Na_2SO_4(s), filtered, and concentrated *in vacuo*. The resulting mixture was purified with silica-gel flash column chromatography to provide compounds **2**. Spectroscopy data of representative compounds **2a**−**2d** are in the Supporting Information.

General Synthetic Procedure for Compound 3. To a solution of 4-fluoro-2-nitrobenzoic acid in DCM, isobutylene (2.0 equiv) and H_2SO_4 (cat.) were added at −78 °C. The reaction mixture was stirred at room temperature. After the completion of reaction monitored by TLC, the reaction mixture was diluted with DCM, washed with saturated $NaHCO_3$(aq) and brine, dried over anhydrous Na_2SO_4(s), filtered, and concentrated *in vacuo*. The residue was purified with silica-gel flash column chromatography to provide *tert*-butyl 4-fluoro-2-nitrobenzoate. Then, to a solution of *tert*-butyl 4-fluoro-2-nitrobenzoate in DMF, amino acid (1.5 equiv) and DIPEA (2.0 equiv) were added, and the reaction mixture was stirred at 50 °C. After the completion of reaction monitored by TLC, the reaction mixture was concentrated *in vacuo*. The residue was purified with silica-gel flash column chromatography to provide compounds **3**. Spectroscopy data of representative compounds **3**{**1**}−**3**{**5**} are in the Supporting Information.

General Synthetic Procedure for Compound 4. To a solution of compound **3** (1.3 equiv) and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC, 3 equiv) in DCM, compound **2** was added. The reaction mixture was stirred at room temperature. After the completion of the reaction monitored by TLC, the reaction mixture was diluted with DCM, washed with saturated $NaHCO_3$(aq) and brine, dried over anhydrous Na_2SO_4(s), filtered, and concentrated *in vacuo*. Without any further purification, the resulting residue, intermediate **A**, was treated with neat formic acid. After stirring at room temperature for 1 h, the reaction mixture was condensed under reduced pressure and purified with silica-gel flash column chromatography to provide compounds **4**. Spectroscopy data of representative compounds **4a**−**4d** are in the Supporting Information.

General Synthetic Procedure for Compound 5. To a solution of compound **4** in DCM, morpholine (3.0 equiv), EDC (3.0 equiv), and DMAP (cat.) were added, and the reaction mixture was stirred at room temperature. After the reaction completion monitored by TLC, the reaction mixture was diluted with DCM, washed with saturated NH_4Cl(aq) and brine, dried over anhydrous Na_2SO_4(s), filtered, and concentrated *in vacuo*. The residue was purified with silica-gel flash column chromatography to provide compounds **5**. Spectroscopy data of representative compounds **5a**−**5d** are in the Supporting Information.

General Synthetic Procedure for Compound 7. To a solution of 4-(aminomethyl)phenol in DCM, 2,2-dimethoxyacetaldehyde (2.0 equiv) and anhydrous Na_2SO_4(s) (30 equiv) were added, and the reaction mixture was stirred at room temperature for 3 h. After the completion of reaction monitored by TLC, the reaction mixture was stopped by filtration and concentrated *in vacuo*. The residue was dissolved in anhydrous THF, and Grignard reagent (3.0 equiv) was added to this solution at 0 °C under argon atmosphere. The reaction mixture was stirred at room temperature for 1 h. After the completion of reaction monitored by TLC, the reaction mixture was quenched by the addition of methanol and concentrated *in vacuo*. The residue was diluted with DCM, washed with saturated NH_4Cl(aq) and brine, dried over anhydrous Na_2SO_4(s), filtered, and concentrated *in vacuo*. For the activation of the fluorous tag, to a solution of diisopropyl-(1*H*,1*H*,2*H*,2*H*-perfluorododecyl)silane (1.5 equiv) in anhydrous DCM, trifluoromethanesulfonic acid (2.0 equiv) was added at 0 °C under an argon atmosphere and stirred at room temperature for 15 h. Then, to the solution of the activated fluorous tag, the resulting amine (1.0 equiv) in anhydrous DCM was added in the presence of 2,6-lutidine (4.0 equiv) and stirred at room temperature for 2 h. After the completion of reaction monitored by TLC, the reaction mixture was diluted with DCM, and the organic layer was washed with saturated $NaHCO_3$(aq) and brine and dried over anhydrous Na_2SO_4(s). The resulting mixture was filtered, concentrated *in vacuo*, and purified with silica-gel flash column chromatography to provide compounds **7**. Spectroscopy data of representative compounds **7a**−**7d** are in the Supporting Information.

General Synthetic Procedure for Compound 9. To a solution of compound **3** (1.3 equiv) and EDC (3.0 equiv) in 1,2-dichloroethane (DCE), compound **7** was added at room temperature in the absence of a base and catalyst. The reaction mixture was stirred at 50 °C. After the completion of reaction monitored by TLC, the reaction mixture was concentrated *in vacuo*. The resulting residue was redissolved in a small amount of DMF and purified by fluorous tag-based solid-phase extraction (F-SPE) to provide intermediate **A**. To a solution of intermediate **A** in DCM (5 mL), neat formic acid (45 mL) was added. The reaction mixture was stirred at room temperature for 1 h. After the completion of reaction monitored by TLC, formic acid was removed under reduced pressure. The residue was purified by F-SPE and subsequent silica-gel flash column chromatography to provide compounds **8** with a fluorous tag. The fluorous tag in compound **8** was removed by treatment with a HF/pyridine/THF (5/5/90) solution (1.0 mL) for 2 h at room temperature, followed by the addition of ethoxytrimethylsilane (TMSOEt, 1.0 mL) to quench an excess amount of HF in solution. The resulting reaction mixture was concentrated *in vacuo* using a GeneVac centrifugal evaporator. The residue was dissolved in MeOH (0.2 mL) and purified by F-SPE. The desired untagged product **9** was collected in the MeOH/H_2O (4:1, v/v) and concentrated *in vacuo* using a GeneVac centrifugal evaporator. After lyophilization of the cleaved product in acetonitrile/water (1:1, v/v), the final products **9** were obtained in powder and analyzed by LC/MS. Spectroscopy data of representative final compounds **9**{a,1} **9**{a,2}, **9**{a,3}, **9**{c,5}, and **9**{d,1} are in the Supporting Information.

General Synthetic Procedure for Compound 10. To a solution of compound **8**, HATU (3.0 equiv), and DIPEA (2.0 equiv) in DMF, secondary or primary amine (5.0 equiv) was added as an R_5 substituent. The reaction mixture was stirred at room temperature. After completion of the reaction monitored by TLC, the reaction mixture was directly purified by F-SPE without condensation to provide intermediates **C**. The fluorous tag in intermediate **C** was removed by the treatment with HF/pyridine/THF (5/5/90) solution (1.0 mL) for 2 h at room temperature, followed by the addition of ethoxytrimethylsilane (TMSOEt, 1.0 mL) to quench an excess amount of HF in solution. The resulting reaction mixture was concentrated *in vacuo* using a GeneVac centrifugal evaporator. The residue was dissolved in MeOH (0.2 mL) and purified by F-SPE. The desired untagged product **10** was collected in MeOH/H_2O

(4:1, v/v) and concentrated *in vacuo* using a GeneVac centrifugal evaporator. After lyophilization of the cleaved product in acetonitrile/water (1:1, v,v), the final products **10** weer obtained in powder and analyzed by LC/MS. Spectroscopy data of representative final compounds **10**{a,2,3} **10**{b,3,6}, **10**{c,3,1}, **10**{c,5,6}, and **10**{d,1,3} are in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

Characterization for intermediates and representative compounds, tri-substituted Δ^5-2-oxopiperazine as γ-turn mimetic, principal component analysis (PCA) of Δ^5-2-oxopiperazine library, copies of ^{1}H and ^{13}C NMR spectra, PDA based LC/MS data for representative compounds, and PDA based LC/MS analysis data for library compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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