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Facile synthesis of benzamides to mimic an α-helix

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Abstract—A new α-helix mimetic was designed by using a benzamide as a rigid scaffold. It presents three functional groups corresponding to side chains of amino acids found at the *i*, *i* + 4, and *i* + 7 positions of an ideal α-helix, which are displayed on the same helical face. Its efficient synthesis was achieved by employing simple alkylation and amidation reactions which can be easily adapted for solid-phase synthesis. As a result, two tris-benzamides were produced to mimic two helical regions found in a peptide hormone, glucagon.

An α-helix is one of the most common structural motifs in proteins and widely used for diverse functions including recognition of other proteins.[1] The formation of protein–protein complex is essential for numerous regulatory processes in cells and paramount for survival. Thus, modulation of the protein–protein interaction is extremely valuable to comprehending biochemical pathways and eventually may lead to develop new therapeutic candidates.[2–5] However, short peptide segments corresponding to helical regions of proteins have difficulty binding to their target proteins because they tend to adopt significantly less organized conformations in solution.[6] Several approaches have been pursued to stabilize α-helices, including making a non-covalent interaction or a covalent bond between side chains of amino acid residues as well as incorporating unnatural amino acids.[7–12]

Another approach is to develop nonpeptidic α-helix mimetics. While a number of amino acids are required to construct a highly ordered, helical structure, it is often found that side chains on one face of a helix are mainly responsible for the interaction with its target proteins. For the development of α-helix mimetics, it is thereby important to retain characteristic structural features of a helix, and only a handful of α-helix mimetics have been created using rigid and pre-organized scaffolds[13–18] (Fig. 1).

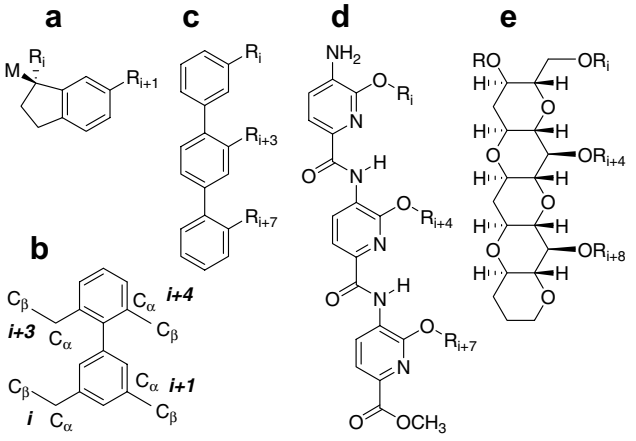

Figure 1. Structures of α-helix mimetics reported previously. (a) Indane; (b) biphenyl; (c) terphenyl; (d) tris-pyridylamide; (e) polycyclic ether.

1,1,6-Trisubstituted indanes (Fig. 1a) reported by Willems and co-workers are to represent amino acids at the *i* and *i* + 1 positions of an α-helix, and the designed mimetics of dipeptides (Phe–Phe and Trp–Phe) showed micromolar affinity similar to the original dipeptides in an attempt to mimic tachykinins and other neuropeptide targets.[13] However, the indanes can mimic only two amino acids, thereby are not suitable to cover more residues in a helix. On the other hand, Jacoby suggested 2,6,3′,5′-tetrasubstituted biphenyl analogues (Fig. 1b) to mimic one helical turn.[14] Recently, Hamilton and co-workers have reported scaffolds which can represent two helical turns. Trifunctionalized 3,2′,2″-terphenyl

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derivatives (Fig. 1c) hold side chains located at the *i*, $i+3$, and $i+7$ positions in two helical turns and are found to be potent antagonists against calmodulin[15] and inhibitors to disrupt p53/MDM2 complex,[16] demonstrating the potential use of α-helix mimetics as therapeutics. A tris-pyridylamide scaffold (Fig. 1d) was also designed to display side chains found at the *i*, $i+4$, and $i+7$ positions to inhibit the formation of Bak BH3/Bcl-xL complex.[17] In addition, ladder-like polycyclic ethers (Fig. 1e) were developed by Hirama and co-workers to mimic the *i*, $i+4$, and $i+8$ positions in a helix.[18]

In this study, we have focused on developing a new α-helix mimetic using a benzamide as a scaffold. Substitution on a tris-benzamide structure allows placement of three functional groups corresponding to the side chains of amino acids found at the *i*, $i+4$, and $i+7$ positions of an ideal α-helix, representing one helical face. Despite its structural similarity to the tris-pyridylamide, the synthesis of the tris-benzamides can be easily carried out (vide infra), and the high synthetic efficiency will be quite valuable for facile production of a large number of derivatives. Achieving high molecular diversity is often necessary in drug discovery since there is always a possibility of trial and error; thus the synthetic efficiency, such as high yields, easy derivatization, ready availability of starting materials, and synthetic chemistries suitable for automation, should be considered as an important factor when developing mimetics.

In addition, the tris-benzamide possesses slightly higher conformational flexibility compared to the tris-pyridylamide due to the lack of a hydrogen bond between an amide proton and a nitrogen on a pyridine ring of the tris-pyridylamide. This hydrogen bond as well as a hydrogen bond between the amide proton and an oxygen in an adjacent ether linkage, significantly rigidifies the tris-pyridylamide as a flat structure, and places its three functional groups on a straight line. This spatial arrangement by the tris-pyridylamide does not properly represent the *i*, $i+4$, and $i+7$ positions in a helix and may result in a weak binding affinity for a protein, Bcl-xL.[17] Although high degree of structural rigidity is prerequisite for a scaffold of a mimetic, small degree of flexibility is also necessary to optimally interact with target proteins that change their structures dynamically.

To demonstrate its α-helix mimicry, computer modeling of a tris-benzamide with the attachment of three functional groups (R_{1-3}) was carried out using MacroModel (version 9, Schrödinger, New York, NY).[19] A Monte Carlo conformational search (5000 steps) was conducted using a MM3 force field[20] implemented into the software. The energy-minimized structure of the lowest energy conformation showed that all three functional groups in the tris-benzamide mimetic are found to be well overlaid over the corresponding side chains of an ideal α-helix. (Fig. 2).

The synthesis of tris-benzamides starts from an alkylation reaction, placing a functional group corresponding to an amino acid at the *i* position of a helix. The hydroxyl group in methyl 4-(*t*-butoxycarbonylamino)-3-hydroxybenzoate (**1**), which was readily prepared from a commercially available 4-amino-3-hydroxybenzoic acid, was reacted with a series of alkyl halides in the presence of a base as described in Table 1. Several bases and solvents were examined to optimize the reaction condition, and the use of NaH in DMF at room temperature resulted in the highest yield. Sodium methoxide also provided comparable yield as NaH, whereas K_2CO_3 and a hindered organic base, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) were found to be inefficient. As a solvent, DMF appears to be more effective than THF since the benzylation in THF required refluxing even with the most efficient base found for the reaction (NaH), whereas DMF provided higher yield at much lower ambient temperature. The alkylation reaction was also carried out with various alkyl halides under the optimized reaction condition, resulting in the desired products in high yield (70–80%). Methyl and benzyl halides mimicking Ala and Phe, respectively,

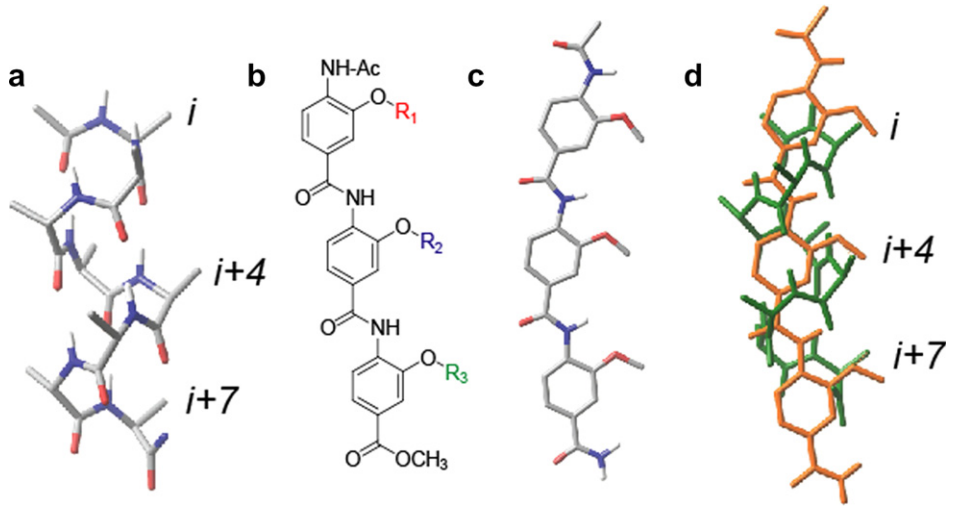
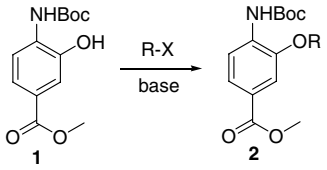
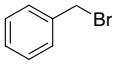
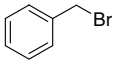
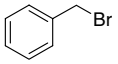
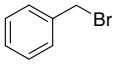
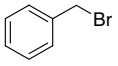
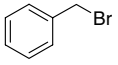
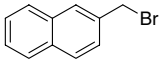


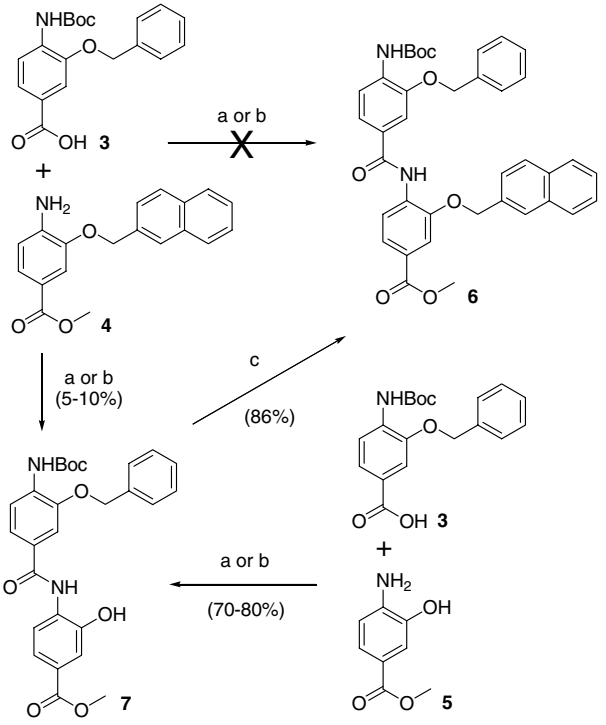
Figure 2. Structures of an α-helix mimetic using a tris-benzamide scaffold. (a) α-Helix; (b) tris-benzamide; (c) energy-minimized structure of a tris-benzamide; (d) superimposition of a tris-benzamide (orange) to an ideal α-helix (green).

Table 1. Alkylation of the 3-hydroxyl group of benzoate (**1**)



Product	R–X	Reaction condition	Yield[a] (%)
2a		K_2CO_3 (1.2 equiv), acetone, reflux, 24 h	33
2a		NaH (1.1 equiv), DMF, rt, 1.5 h	83
2a		NaH (1.1 equiv), THF, reflux, 2.5 h	53
2a		NaOMe (1.2 equiv), DMF, rt, 1.5 h	73
2a		NaOMe (1.2 equiv), THF, reflux, 2.5 h	72
2a		DBU (5 equiv), DMF, rt, 12 h	19
2b	CH_3I	NaH (1.1 equiv), DMF, rt, 1.5 h	86
2c		NaH (1.1 equiv), DMF, rt, 24 h	78
2d		NaH (1.1 equiv), DMF, rt, 24 h	73
2e		NaH (1.1 equiv), DMF, rt, 1.5 h	83

[a] Yield was determined after purification by column chromatography.

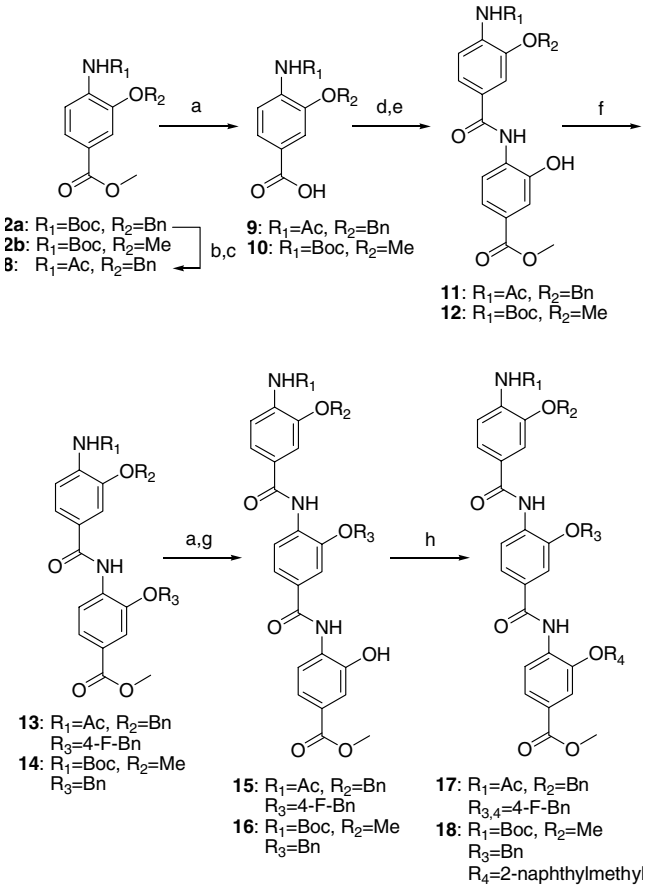


Scheme 1. Formation of an amide bond for the synthesis of dialkoxy-bis-benzamide (**6**). Reagents and conditions: (a) (i) **3**, $SOCl_2$, THF, DMF (cat), 60 °C, 2 h; (ii) **4** or **5**, DIPEA, DCM, 0 °C→rt, 2 h; (b) **4** or **5**, BOP, DIPEA, DCM, rt, 2 h; (c) 2-bromomethylnaphthalene, NaH, DMF, rt, 2 h.

gave slightly better yields compared to aliphatic alkyl halides, such as 2-bromopropane and 2-bromobutane representing Val and Ile, respectively. 2-Naphthylmethyl group was introduced to represent the indole side chain of Trp.

After the alkylation, formation of an amide bond to produce dialkoxy-bis-benzamide (**6**) was attempted (Scheme 1). Free carboxylic acid and amine groups were released by hydrolysis of a methyl ester and removal of an *N*-Boc group from two methyl alkoxybenzoates, respectively. Several coupling reagents were examined including $SOCl_2$, $(COCl)_2$, DCC, EDC, HBTU, BOP, PyBrOP, and TFFH, however all of them failed to produce the desired bis-benzamide (**6**), presumably due to substantially reduced nucleophilicity of the amine and electrophilicity of the benzoic acid as well as steric hindrance caused by the alkoxy group of aniline (**4**). While the starting materials were mostly recovered after the reaction, it is quite interesting that the reaction yielded monoalkylated bis-benzamide (**7**) as a byproduct (5–10% conversion). Hinted by the formation of byproduct (**7**), the coupling reaction was successfully carried out using unalkylated aminobenzoate (**5**) instead of alkylated (**4**). Using $SOCl_2$ or BOP as a coupling reagent, monoalkoxy-bis-benzamide (**7**) was synthesized in high yield (70–80%), and a subsequent alkylation produced the desired bis-benzamide (**6**) possessing two functional groups corresponding to the i and $i+4$ positions in a helix.

For the evaluation of the tris-benzamides as α-helix mimetics, we have synthesized two analogues to mimic α-helical regions found in glucagon. Glucagon is a 29 amino acid-containing peptide hormone and plays a critical role in glucose homeostasis by stimulating glucose release upon binding to its membrane receptor in liver.[21] Therefore, potent glucagon antagonists[22–24] have been pursued as a potential therapeutic candidate for *diabetes mellitus*, and nonpeptidic glucagon receptor antagonists[25–28] have also been reported while most of them turned out to be noncompetitive. Recently, glucagon is found to adopt helical conformations in two separate regions when it binds to the receptor,[29] thus two tris-benzamides were designed to mimic hydrophobic faces of the two helical regions in glucagon. Two tris-benzamides (**17** and **18**) contain benzyl, 4-fluorobenzyl, and 4-fluorobenzyl; and methyl, benzyl, and 2-naphthylmethyl groups as three functional groups which represent Phe,[6] Tyrp,[10] and Tyr;[13] and Ala,[19] Phe,[22] and Trp,[25] respectively. The 4-fluorobenzyl group was employed to substitute the side chain of Tyr because the 4-hydroxybenzyl group was unstable during the synthesis.



Scheme 2. Synthetic scheme to produce two tris-benzamides as α-helix mimetics for glucagon. Reagents and conditions: (a) NaOH/MeOH/THF, 60 °C, 2 h; (b) 20% TFA/DCM, rt, 2 h; (c) Ac_2O, DMAP, rt, 12 h, 97% over two steps (b, c); (d) $SOCl_2$, THF, DMF (cat), 60 °C, 2 h; (e) methyl 4-amino-3-hydroxybenzoate (**5**), DIPEA, DCM/THF, 0 °C→rt, 2 h, 82% (**11**) and 77% (**12**) over three steps (a, d, e); (f) R_3X, NaH, DMF, rt, 2 h, 77% (**13**) and 80% (**14**); (g) **5**, BOP, DIPEA, DCM, rt, 2 h, 74% (**15**) and 77% (**16**) over two steps (a, g); (h) R_4X, NaH, DMF, rt, 2 h, 61% (**17**) and 86% (**18**).

The synthesis of two tris-benzamides (**17** and **18**) is summarized in Scheme 2. After the alkylation of hydroxybenzoate (**1**), methyl ester (**8** or **2b**) was hydrolyzed using NaOH, and methyl 4-amino-3-hydroxybenzoate (**5**) was coupled to benzoic acid (**9** or **10**) using $SOCl_2$, resulting in a bis-benzamide containing one alkyl group (**11** or **12**) corresponding to the *i* position of a helix. The alkylation and coupling reactions were repeated twice to place two other functional groups corresponding to the $i+4$ (or $i+3$) and $i+7$ positions, and the target tris-benzamides (**17** and **18**) were easily synthesized with high yield.

In summary, a new α-helix mimetic was designed using a tris-benzamide as a rigid scaffold, and it mimics one face of a helix, displaying functional groups found at the *i*, $i+4$, and $i+7$ positions. Its facile and rapid synthesis was achieved by using simple alkylation and amide bond-formation reactions which can be easily adapted for solid-phase synthesis to construct a combinatorial library of α-helix mimetics. To demonstrate the proof of concept, two tris-benzamides were prepared to represent two helical regions in glucagon and will be evaluated for receptor binding and biological activity.

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Supplementary data

Available for experimental procedures and spectral characterization. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.03.108.

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