

Peptidomimetics of Helical Protein Surfaces Targeting the *E. coli* CheY Response Regulator.

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Introduction

The introduction of antibiotics in the 1930's heralded a new era to treat infections; however, by the 1950's the emergence of pathogenic strains resistant to therapeutics was already apparent [1]. Today, a new era has emerged in which our existing armory of antibiotics is inadequate to combat microbial infections.

Historically, drug discovery efforts have aimed at developing broad-spectrum agents that arrest infection by pathogen toxicity or growth inhibition [1]. An alternative approach is through altering virulence-related activities, such as chemotaxis [2]. Chemotaxis enables the pathogen to direct their movements according to chemical gradients within their environment. Modification of endogenous chemotactic ability of microorganisms by pharmaceutical agents has the potential to limit the spread of infectious diseases.

Chemotaxis within bacteria is best understood in the two-component regulatory system, where CheA is the histidine kinase, and CheY the response regulator [3]. Phosphorylated CheY binds to the flagellar motor; specifically, the FliM protein in the basal body of the flagellar motor that extends into the interior of the cell. Binding CheY phosphate causes the rotation of the flagellar motor to change the sense of rotation from counterclockwise to clockwise. Dephosphorylation, by CheY autophosphatase activity, or by the phosphatase CheZ, and dissociation from FliM reverts this action. The CheY domain is conserved among all response regulators and is the central hub for protein-protein interactions on its C-terminal α 4- β 5- α 5 surface [4]. Therefore, if we interfere with binding to CheY, we should identify new anti-pathogenic compounds. It was our intent to interdict, in general, the pathways responsible for virulence in pathogens.

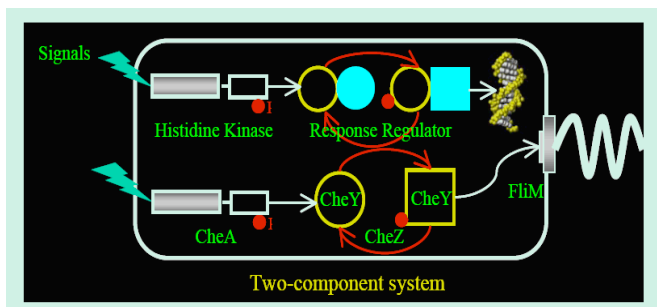


Fig. 1. Schematic diagram of bacterial two-component response systems including the prototypical CheY/flagellar control system as well as other response-regulators that control gene expression

Results and Discussion

Design and Target Synthesis. The CheA/FliM/CheZ interfaces all employ a helix to mediate the CheY-interaction, although the orientation of the CheA helix is almost perpendicular to that of FliM/CheZ [5]. N-terminal FliM and C-terminal CheZ peptides are sufficient for binding to the CheY cleft. This structural information combined with the fact that the CheY/FliM interface is downstream of the adaptation response in the chemotaxis two-component regulatory network indicated these helices as possible targets for helix peptidomimetics.

The α -helix is a common secondary structural motif within proteins and peptides, often associated with protein/protein and protein/nucleic acid interactions [6, 7]. Efforts to mimic these surfaces with organic scaffolds such as terphenyl aromatics have met with considerable success [8-11].

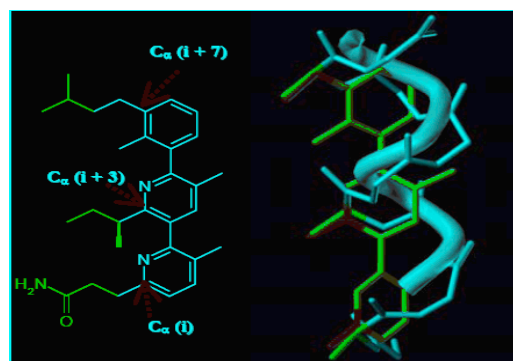


Fig. 2 Helical peptidomimetic designed to mimic binding surface of FliM (8-16) and bind to CheY to displace CheA, CheZ and FliM.

Our syntheses of helical peptidomimetic targets as a potential CheY inhibitor involves both the approaches of Jacoby [8] and Hamilton [9-11]. We decided on an approach where a pair of aromatic rings (Ring A and Ring B) were synthesized independently and then a Pd-based coupling reaction was used to combine the fragments (Fig. 3). From modeling studies, we decided upon a 2-methyl benzyl analog and a pyridine analog as the core heterobicyclic. The methyl group and the lone pair of the electrons of the nitrogen stabilized a twist in the core heterobicyclic. Pyridine also has the added benefit of increasing solubility over biphenyl derivatives.

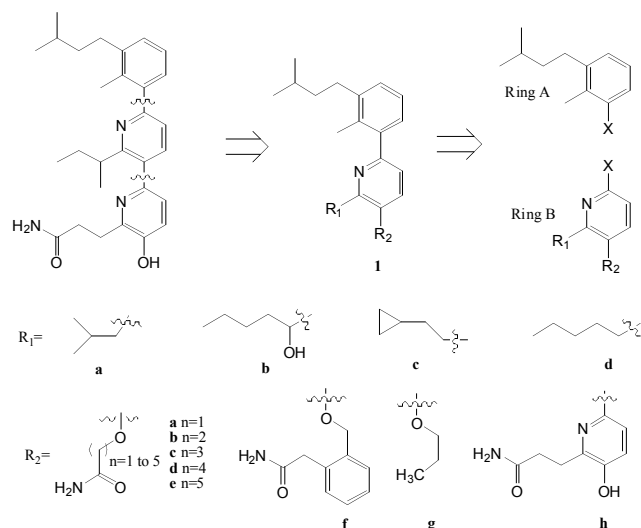


Fig. 3. Disconnection Approach and Target Library.

Synthesis of Phenyl Aromatic A. Arylstannanes **5a,b** or the arylboronic acids **5c** were easily prepared using simple starting materials (Fig. 4). Simply, the dibromo species **3a** (commercially available) or **3b** (synthesized in three steps) [12] was reacted with isobutylmagnesium bromide. A major side product of cross-coupling reactions of Grignard reagents was the possibility of trans-metallation between isopropyl magnesium bromide and the benzyl bromide derivative **3a**, **3b**. This was then followed by alkylation with a second equivalence of **3a**, **3b** to form a 1,2-diphenyl ethane derivative. To increase the yield of the wanted product and to decrease this dimerization, we investigated cross-coupling reactions of Grignard reagents of aryl halides in the presence of various transition metal catalysts. For our purposes, we decided upon Li_2CuCl_4 to help increase the yield substantially ($\text{R} = \text{H}$, 81 %, $\text{R} = \text{CH}_3$, 61 %) [13]. Without the catalysts, we obtained (<20 %) of the desired materials.

At this point, a decision to perform the Suzuki or Stille coupling to give our wanted template was needed. Fisher observed that the boronic moiety to the nitrogen of the pyridine ring was unstable (when performing Suzuki couplings) [14]. This was reconfirmed by several other authors who noted a slight deformation of the boronic ester moiety due to the nitrogen [15-17]. Often, attempts to isolate the requisite pyridin-2-ylborane adducts have always been problematic [16]; a possible consequence of the C–B bond being longer and more fragile in the pyridin-2-yl derivatives. We decided to investigate both the Stille and Suzuki reactions and synthesized several organostannyl and boronic intermediates **5a-c**.

Synthesis of Pyridine Aromatic Ring B. We initiated the synthesis of the pyridine Ring B (Fig. 5) starting with 3-hydroxy-pyridine **5** (commercially available) followed by iodination and SEM protection to give **7** (Scheme 2) [19, 20]. Hasseberg et al. [20] showed that alkylation was easily achieved at the 2-position of the pyridine ring of a dihalogenated species. During alkylation, there exists coordination between the lithium cation and the oxygen of the SEM group **7** stabilizing the reactive intermediate.

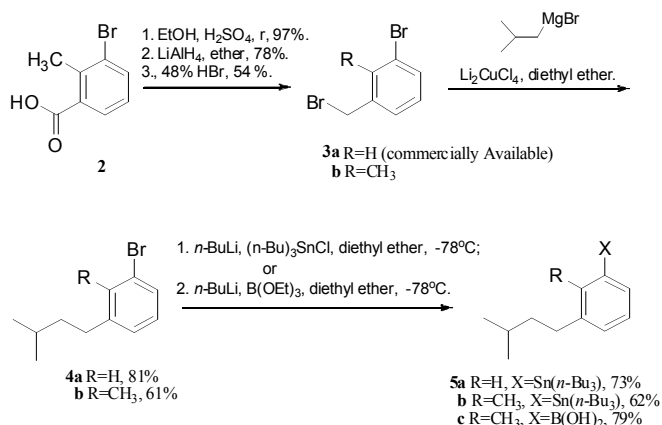


Fig. 4. Synthesis of Phenyl Aromatic Ring A.

However, the authors showed only one case of alkylation (methylation). Repeating this work, we easily achieved the wanted formylation to give the corresponding aldehyde which was subsequently protected using ethylene glycol to give **9a,b**. Note that if we changed the solvent slightly for alkylation (THF vs diethyl ether), it was possible to achieve deprotonation at the 6-position rather than metal exchange at the 2-position. This provided us with a possible new set of functionalized sterically hindered aromatics that could be incorporated into helix mimetics.

For the Stille/Suzuki cross coupling we drew upon a method described recently by Baldwin et al. [21]. Baldwin observed, the $\text{PdCl}_2/\text{PtBu}_3$ catalytic system with copper(I)iodide and cesium fluoride in DMF was most effective for coupling aryl bromides, while palladium catalysts in combination with copper(I) iodide and cesium fluoride were optimal when coupling iodides and triflates. It was assumed that transmetalation between the organostannane and CuI was in equilibrium. Therefore, the removal of the Bu_3SnI by-product as insoluble Bu_3SnF favors the formation of the more reactive organocopper species, resulting in enhancement of this reaction.

We decided to investigate the cross-coupling reactions between Ring A and Ring B to give **10a,b**. The yields were significantly higher when we performed the Stille or Suzuki coupling between the organostannane or the organoboronic acid of ring A and the iodide of ring B.

The next step required the removal of the protecting groups (the acetal and the SEM group). A test reaction for SEM deprotection was carried out using the iodide **9a** with TBAF. The conditions for complete removal required heating with HMPA as solvent as reported by Barua et al. [22]. Interestingly, also removed was the acetal with only small amounts of the desired alcohol obtained. We applied the same conditions to **10a**, but only the SEM group was removed. We attempted to remove the acetal using acid hydrolysis; however, this caused decomposition of our compound with no detectable product, even under dilute acid conditions. We concluded a milder non-evasive method for acetal removal was required. We selected conditions reported by Marcantoni and Nobili [23] using lanthanide cerium chloride. A search of the literature revealed no reference of using cerium chloride to remove the SEM protecting group. This group can sometimes be problematic when attached to phenols and should prove

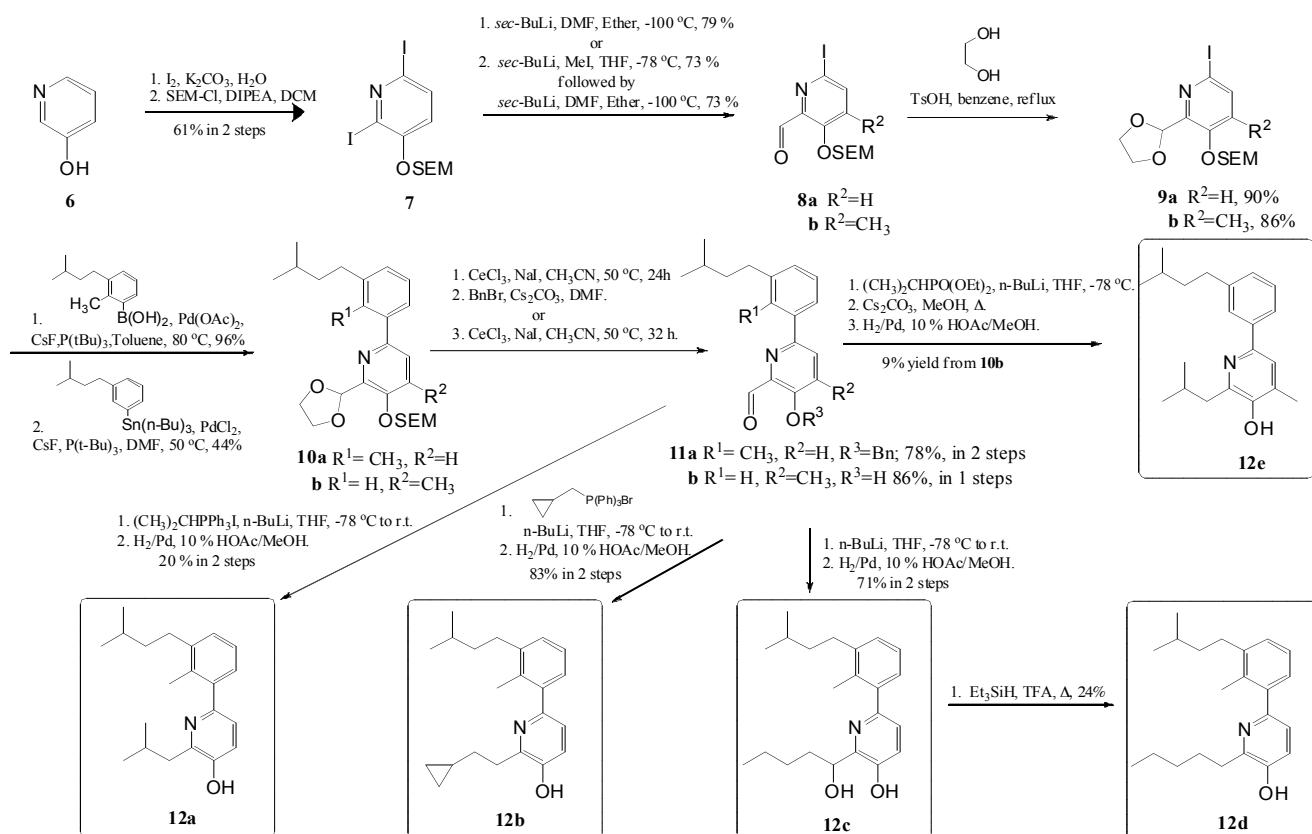


Fig. 5. Synthesis of Several Scaffolds.

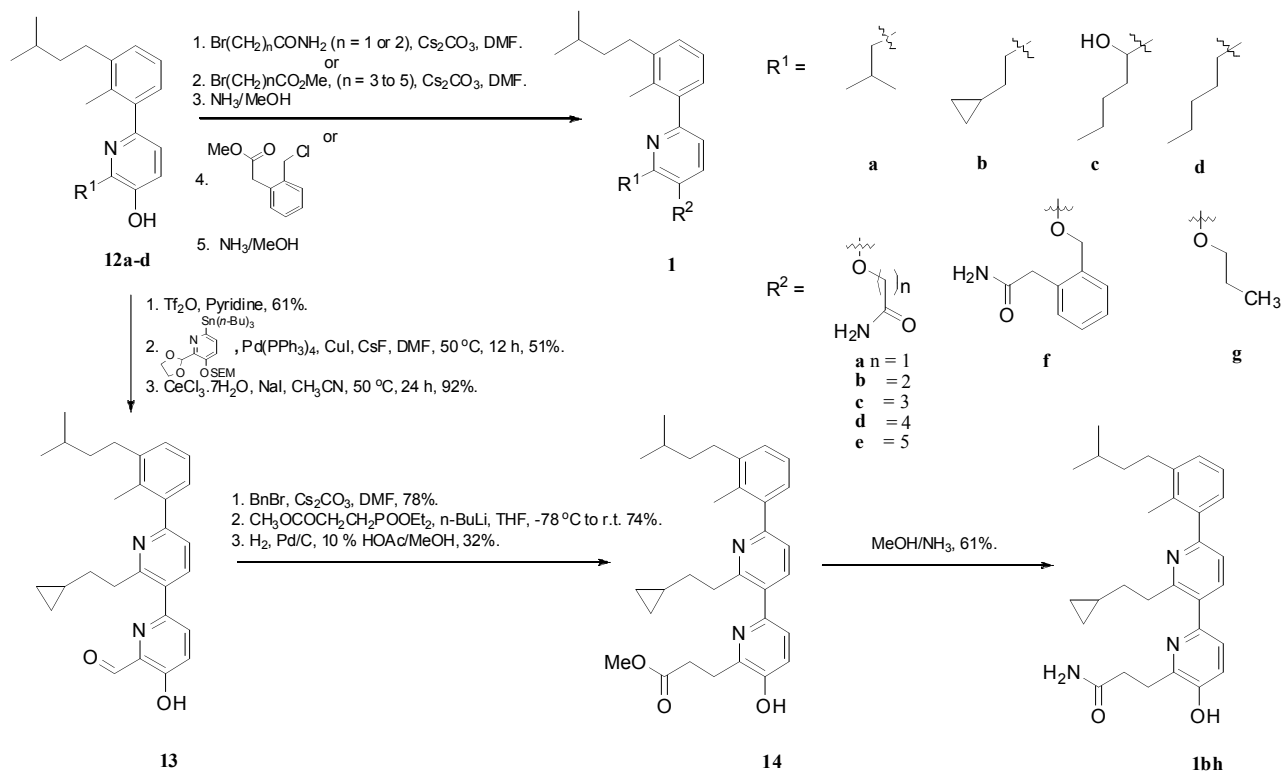


Fig. 6. Library Synthesis.

useful for future synthesis. In our reaction the SEM group was removed quickly (LC-MS), while the acetal cleavage required overnight heating (45° C). The resultant alcohol **11a** was subsequently protected as the benzyl ether followed by a Wittig reaction or alkylation to produce a series of templates. The alcohol was protected since Horner-Emmons conditions on **11b** produced a stable betaine intermediate that required strenuous conditions to convert to the alkene **12e**. The benzyl protecting group was used since the next step required the reduction of the double bond, and thus the benzyl and allyl protecting group reduction could be performed simultaneously. Finally the alcohol **12c** was dehydroxylated using refluxing TFA/Et₃SiH [24] to give **12d** in 22 % yield.

Library Synthesis. Based on this success, we synthesized a number of compounds for assay as inhibitors of CheY/Flim binding. The initial library generated is shown in Fig. 6 and was formed by simple alkylation of the phenyl-pyrid-3-ol moiety with a series of alkyl halides followed by ammonolysis when required. The only difficulty was with the purification of analogs containing the propionamide (n = 2) **1ab**, **1bb**, **1cb**, **1db**. Under aqueous acidic conditions (reverse phase HPLC conditions), the resultant library member decomposed to the starting material and the allyl amide equivalent. This side reaction could be circumvented by performing purifications under non-aqueous conditions.

A final helix mimetic was synthesized, having a phenyl-dipyridal scaffold. We wanted to develop such a scaffold as depicted in Figure 2. for several reasons, i) to show the synthesis was easily adaptable, ii) to increase the order of complexity and iii) to restrict the conformation of the glutamine side chain within our mimetic. The wanted α -helix mimetic was synthesized stepwise by initially producing the triflate followed by the Stille reaction with **10a**. Deprotection with CeCl₃·7H₂O, and an iodine salt catalyst, benzylation and a Horner-Emmons reaction produced the allyl intermediate. Hydrogenation reduced the double bond in conjunction with the removal of the benzyl ether to give **14**. Ammonolysis subsequently gave our wanted compound **1bh**. All synthesized compounds are currently being tested by several bioassays. Results will be reported elsewhere.

Conclusion. One of the most difficult tasks for the medicinal chemist is finding new leads that mimic the discontinuous surface of proteins while meeting several parameters including ADME profile, molecular weight and pharmacokinetic parameters. Privileged structures represent an ideal source of lead compounds, already possessing many desirable biological characteristics [25, 26]. The term 'privileged structure' has gained prominence in the literature since it was first introduced some fifteen years ago. However, by definition privileged structures are not structures in their own right, as they usually comprise only the core subsection of any molecule. For the medicinal chemists, the true utility of privileged structures is the ability to synthesize one library based upon a single core scaffold and screen it against a variety of different receptors, yielding several active compounds against different biological targets. The biphenyl framework is

without doubt a privileged structure and such is found in 4.3 % of all known drugs.

In this project, we have used an analogous framework to synthesize a small solution-based library of helix peptidomimetics targeting the CheY response regulator. The framework: phenyl-pyridal, phenyl-dipyridal enables mimicking discontinuous surfaces, has a lower log P than the biphenyl analogs and is expected to have useful pharmacokinetic characteristics. Finally, the overall synthesis is both highly convergent and high yielding.

Acknowledgments

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