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Synthesis of the Phenylpyridal Scaffold as a Helical Peptide Mimetic

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Abstract

Phenylpyridal- and phenyldipyridal-based scaffolds have been designed and synthesised as novel helical peptide mimetics. The synthesis required optimisation and selective alkylation in producing 2, 6 functionalised 3-hydroxypyridine derivatives for a convergent scheme. The pyridine analogs were coupled by a series of Suzuki/Stille types cross-coupling reactions. A series of biaryl and ter-aryl substituted heterocycles were produced. The synthetic approach was concise and high yielding allowing large variability at the wanted side-chain attachment points. A number of compounds were synthesised to show the versatility of the strategy.

Keywords

helical structures; proteomimetics; biaryls; cross coupling; alkylation

Introduction

In proteins, helices are frequently found to act as structural scaffolds, orienting important side chains for molecular recognition.^[1-3] Networks of intra-residue hydrogen bonds stabilize the helical backbone. Hydrogen bonds are flexible and compensatory, so helical backbones are not rigid. Consequently, the residues projected by a helix are also flexible in their relative orientations. Rigidifying the helical backbone may improve the thermodynamics of binding and optimize the physicochemical profile of a helical peptidomimetics.^[2]

Organic scaffolds opens the door to stabilizing helical mimetics with various strategies beyond the hydrogen bond.^[2-4] The challenge of organic helix mimetics is to design a scaffold that orients side-chain R-groups so that C_α-C_β vectors are projected as in the target helix. A statistical population of helical conformations from high-resolution X-ray crystallographic model structures was found to populate a single smooth distribution centered at ($\phi = -62$, $\psi = -43$) in Ramachandran dihedral coordinates.^[5] Thus, a good organic helical scaffold should reproduce the same C_α-C_β projection vectors for orientation of the side chains involved in helix recognition.

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Jacoby suggested 2,6,3'5'-substituted biphenyls as better than allenes, alkylidene cycloalkanes and spiranes as helical mimetics of side-chain positions i , $i+1$, $i+3$ and $i+4$.^[6] The Hamilton group suggested the terphenyl scaffold to mimic positions i , $i+1$, $i+3$ and $i+4$.^[7] More recently, the Schepartz group has suggested helical β -peptides as helical peptidomimetics and demonstrated their utility in the p53/hdm2 system.^[8] Kelso et al. have used a motif of HXXXH complexed with Pd(en)²⁺ to preorganize peptides into helices.^[9] Taylor has advocated the use of side-chain lactams for a similar purpose.^[10] Other helical mimetics, based on stereochemical modification of the peptide C α with a methyl group and "stapled" hydrocarbon side chains, fall outside the domain of organic helical scaffolds.

Helical scaffolds were designed and evaluated *in silico* using molecular dynamics and *ab initio* methods.^[2] Analysis revealed that aromatic scaffolds such as Jacoby's biphenyl scaffold^[6] and Hamilton's terphenyl scaffolds^[7,11] and the phenyldipyridal scaffold discussed herein are fundamentally limited in accurately mimicking the torsional values of C α -C β "launch" vectors (Csp3-Csp3 bonds) of a native helical peptide, due to their Csp2-Csp3 nature^[4,12]

Consequently, these scaffolds can accurately mimic side-chain surfaces only when they are long and flexible and, therefore, able to compensate for such torsional preferences in the launch vector. Indeed, Hamilton's group corroborated the biological utility of the terphenyl scaffolds as helical-mimetic ligands, but only for flexible hydrocarbon side chains.

Our molecular modeling suggested that steric conflict between proximal hydrogens causes the angular twist in the biphenyl and terphenyl scaffolds. The pyridine unit replaces the steric bulk of a hydrogen with a smaller lone pair of electrons of the nitrogen. Thus, the potential energy surface is modified so that the conjugated ring systems populate more coplanar angles, and C α -C β vectors are more biased towards the helical-mimetic configuration.

The proposed synthesis of the phenylpyridal helical peptidomimetic involves both approaches of Jacoby^[6] and Hamilton.^[7,11] Interestingly, a similar approach by Hamilton, produced the ter-pyridal scaffold in a concise synthetic approach as alpha helix mimetics.^[12] However the synthesis showed little flexibility to change the side chains attached to the core scaffold. To demonstrate our strategy, a triaryl surface Gln - Leu mimetic - Leu (compound I) was synthesised. The aromatic rings were synthesised independently followed by convergent Pd-based coupling reactions to combine the fragments.

Results and Discussion

Synthesis of Ring A

Arylstannanes **5a,b**, or the arylboronic acids **5c**, can be easily prepared using simple starting materials (Scheme 1).^[13] The dibromo species **3a** or **3b** was reacted with isobutylmagnesium bromide. A major side product of cross-coupling reactions of Grignard reagents is trans-metallation between isobutyl magnesium bromide and the benzyl bromide derivative **3a**, **3b**. This was then followed by alkylation with a second equivalent 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 in the presence of various transition-metal catalysts. For our purposes, Li₂CuCl₄ increased the yield^[14] substantially (R = H, 81%; R = CH₃, 61%). Without the catalysts, we obtained (<20%) of the wanted materials. The final step required trans-metallation to give the desired products.

Preliminary studies on functionalizing pyridines

The B and C rings (Y = OSEM, Scheme 2) were prepared using 3-hydroxypyridine **6** followed by bromination (Scheme 2). Hasseberg et al. showed that when PG = OSEM, (B, C ring, Figure 2), alkylation is easily achieved at the desired ortho position to the OSEM group.^[15] It is thought that during alkylation coordination between the lithium cation and the oxygen of the SEM group **7** stabilizes the reactive intermediate. Alkylation then proceeds solely at the 2 position of the pyridine ring; the authors showed, however, only one case of alkylation (methylation). Koch et al. investigated halogenations of 3-hydroxypyridines and observed low yields for dibrominations, but high yields for diiodinations.^[16]

The initial dibromination of 3-hydroxypyridine under the reported procedures gave a mixture of monobrominated and dibrominated products that proved difficult to separate. The best yield obtained was 17% and required a long-gradient chromatography followed by several recrystallizations. The ratio by NMR was approximately 2:2:1, 2-bromopyridine : 2,4 dibromopyridine : 2,6 dibromopyridine.^[15,16] For iodination we obtained quantitative yield for the desired 2,6 derivative, and found that we can obtain selective monobrominated, iodinated products as well. Protection of the alcohol with the metallation-directing SEM protecting group^[16] then afforded **7a-c**.

We next examined the regioselectivity of alkylation by simply treating the lithium derivative of **7a** with NH₄Cl giving rise to the monobrominated species. An inspection of the coupling patterns in the aromatic region of the NMR spectra revealed two protons that signified *ortho* couplings (J = 8.8 Hz), while one of these protons also contained a *meta*-coupling pattern to a third proton (J = 2.7 Hz).

As our goal was to generate diverse libraries of α -helix mimetics, we required a high-yielding route that allowed multiple fictionalizations. We reinvestigated this system with a series of nucleophiles to determine the generality of the procedure (Scheme 2, Table 1). Simple alkylations of the dihalogenated pyridine with several nucleophiles (allyl bromide and methyl iodide) were successful. For example treatment of dibromopyridine with allyl bromide gave an 85% yield.

We failed, however, when using other non-activated electrophiles. The lithium salt of **7** was found to be stable at -78 °C existing for long periods of time in the presence of a non-activated alkyl halide. Upon quenching, we obtain the monobrominated species as the major product. We can view the desired pyridine moiety as a 1,2,3,4 tetra-substituted aromatic (2 halogens, an ether and a lone pair). Therefore, alkylation proceeds for the non-hindered cases, methyl iodide and allyl bromide, where attack at the carbon atom is accessible.

To support this argument, we attempted alkylation with propyl bromide, but this reaction failed. By HPLC we detected <1% of the product. To confirm, we synthesized the propyl species **8d** separately through simple hydrogenation of **8c** for a control for HPLC identification. Hydrogenation in a Parr generator at 1.5 atm for 6 hrs in EtOAc provided the desired material quantitatively. Interestingly, using MeOH in this reaction caused partial removal of the SEM group. This low yield agreed with similar results showing that alkylations can be problematic.^[18] Other studies have since showed that copper catalysts/Grignard reagents can substantially increase the respective yields.^[19]

A hypothesis for the problematic alkylation follows. Initially, the desired 2-halogen-lithium exchange occurs in diethyl ether. In THF, we obtain deprotonation at the 4-position. This product can then undergo homo-transmetallation with starting material at higher temperatures >70 °C, followed by a second equilibrium giving rise to the tri-iodo species in

minute amounts. A further possible equilibrium exists which gives the 6-lithio species that is stabilized by the electron-withdrawing effects of the dihalogenated species. At even higher temperatures (close to 0 °C), we also obtained a diene species in small amounts through decomposition of the pyridine system. Alkylation, when using *n*-BuLi as the organic base, to a successful transmetallation versus deprotonation required longer reaction times at -100 °C in anhydrous diethyl ether rather than at -78 °C in THF with non-hindered fairly reactive electrophiles.

We further tested low-temperature alkylation using two different electrophiles (DMF and paraformaldehyde). The desired compounds **8j** and **8i** were obtained in 8% and 79% yields respectively. The low yield of **8j** was anticipated due to slow decomposition of paraformaldehyde to formaldehyde at a higher reaction temperature than the nominated alkylation. This indicated that the lower temperatures were required for alkylation before rearrangement and side-product formation. We tested this hypothesis through methylation of **7c** in two different solvent mixtures (diethyl ether and THF) to obtain **8g** and **8h** respectively in high yield (81% and 83% respectively). The key to successful transmetallation versus the deprotonation reaction was longer reaction times occurring at -100 °C in anhydrous diethyl ether rather than -78 °C in THF with sterically non-cumbersome, highly reactive electrophiles.

Finally, we took **8h** and performed formylation under the standard conditions to obtain **8k**. By the preparation of **8k**, a synthetic route to functionalize pyridine simultaneously at the 2, 3, 4, and 6 positions has been developed. Furthermore, since the iodo group at the 6-position and the hydroxyl group at the 3-position are present, these sites are readily accessible for further chemical modifications. Thus, development of this synthetic methodology provided viable routes to the targeted analogs and demonstrated that one can functionalize easily 4 of the 5 positions available on the pyridine ring.

Synthesis of pyridine-based B and C rings

Synthesis for the final pyridine templates was accomplished using ethylene glycol to form the acetal followed by a second transmetallation (Scheme 3). At this stage the choice was between Suzuki or Stille couplings. Fisher observed that the boronic moiety in the 2-position of pyridine ring was unstable (when performing Suzuki couplings).^[20] This was reconfirmed by several other authors who noted a slight deformation of the boronic ester moiety due to the nitrogen.^[21] As a matter of fact, attempts to isolate the requisite pyridin-2-ylborane adducts have generally been problematic.^[21] The C–B bond length may render it more labile in pyridin-2-yl. We chose to investigate the Stille reaction, and thus synthesized the organostannyl compounds **11a** and **11b**.

For the Stille cross coupling, a method described by Baldwin et al.^[23] was used. Baldwin and co-workers observed that the PdCl₂/PtBu₃ 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 was optimal when coupling iodides and triflates. We decided to test the Stille reactions between Ring A and Ring B. The yields were higher when we performed the Stille coupling between the organostannane of ring A and the iodide of ring B (Table 2). Interestingly, the use of organoboronic acids for Suzuki coupling, gave much higher yields (table 2, entry 3).

The next step required the removal of the protecting groups (both the acetal and the SEM group). A test reaction was carried out using the iodide **9a** with TBAF. The conditions for complete removal of both protecting groups required heating, with the apolar solvent HMPA.^[24] We applied similar conditions to **12a**, but only the SEM group was removed for this phenyl-pyridal compound. We attempted a two step approach to remove the acetal using

acid hydrolysis; however, this caused decomposition of our starting material with no detectable product even under dilute acid conditions. Therefore, a milder method for acetal removal was required.

We selected conditions reported by Marcantoni et al. using cerium chloride in acetonitrile with NaI as catalyst. (Scheme 3)^[25]. These conditions successfully removed the acetal group and, not surprisingly, also removed the SEM protecting group. A search of the literature did not reveal any previous reference for the use of cerium chloride to remove the SEM protecting group. The SEM group can sometimes be problematic when attached to phenols^[24,26], and cerium chloride may be useful for future use in deprotection of the SEM group. In our reaction, the SEM group was removed quickly (analyzed by LC-MS), while the acetal deprotection took longer with mild heating before the aldehyde was obtained.

The alcohol **14** was protected as the benzyl ether followed by either a Wittig reaction or alkylation to produce a series of helix templates. The alcohol was protected, since under the Horner-Wadsworth-Emmons conditions, a stable betaine intermediate was produced. The benzyl protecting group was used since the next step required the reduction of the double bond, and the benzyl group could be removed simultaneously. Dehydroxylation of **15c** using refluxing TFA/Et₃SiH gave **15d** in 22% yield.^[27]

Based on this general route, a number of helix mimetics structurally similar to Jacoby's biphenyl α -helix mimetics were prepared. The initial library generated is shown in Scheme 5 and was formed by simple alkylation of the phenyl-pyrid-3-ol moiety followed by ammonolysis when required. The only difficulty was with purification of analogs containing the propylamide ($n = 2$) **17ab**, **17bb**, **17bc**, **17bd**. Under aqueous acidic conditions (reverse-phase HPLC conditions), the resultant library member decomposed to the starting material and the allyl amide equivalent. This was circumvented by performing purification under non-aqueous conditions

Phenylbipyridal α -helix mimetic

The desired α -helix mimetic was synthesized stepwise by initially producing the triflate followed by the Stille reaction with **11a**. Deprotection with CeCl₃·7H₂O, benzylation and a Horner-Wadsworth-Emmons reaction produced the allyl intermediate with an *E*-orientation **20**. Finally, hydrogenation reduced the double bond and removed the benzyl ether protecting group, followed by ammonolysis to give the targeted compound **1**.

Conclusion

One of the most difficult tasks for the medicinal chemist is finding new leads that mimic the discontinuous interacting surface of proteins while meeting other important parameters including ADME profile, molecular weight and pharmacokinetic parameters. Privileged structures represent an ideal source of lead compounds, as they often already possess many desirable pharmaceutical characteristics.^[28] The term 'privileged structure' has gained prominence in the literature since it was first introduced some fifteen years ago. Privileged structures are by definition, however, generally not structures in their own right, as they usually comprise only the common core scaffold. 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 as 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. The frameworks, phenyl-pyridal and phenyl-dipyridal,

enable mimicking discontinuous surfaces, have a lower log P than the biphenyl analogs and are expected to have useful pharmacokinetic characteristics, including improved solubility relative to other organic helical scaffolds. Finally, the overall synthesis of these helix mimetics is both highly convergent and high yielding

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Synthesis of the proposed α -helix mimetics. **A.** Computational mimicry of the helix overlaid with the ter-aryl analogs. **B.** Disconnection approach in producing the alpha-helix mimetics.

Scheme 1.

Synthesis of Ring A. The compound **3a** was purchased.

Scheme 2.
Alkylation of dihalogenated species

Scheme 3.
Synthesis of Phenylpyridine-based A-B dimer.

Scheme 4.
Library Generation.

Scheme 5.
Synthesis of the tri-aryl (phenyl dipyridal) helix mimetic.

Table 1

Alkylation of the dihalogenated Pyridine Ring.

Entry	Reactant	Electrophile	Product	Yield ^[c]
1		NH ₄ Cl	8a	77% ^[a]
2	7a	MeI	8b	69% ^[a]
3			8c	85% ^[a]
4			8d	<1% ^{[a], [b]}

Entry	Reactant	Electrophile	Product	Yield ^[c]
5	7b	ClSn (nBu) ₃	8e	57% ^[b]
6	8e	DMF	8f	59% ^[a]
7	7c	MeI	8g	81% ^[a]
8	7c	MeI	8h	83% ^[b]
9	7c	(CH ₂ O) _n	8i	8% ^[a]

Entry	Reactant	Electrophile	Product	Yield ^[c]
10		DMF	8j	79% ^[a]
11	8h	DMF	8k	73% ^[a]

^[a] -100 °C, diethyl ether, lithium base

^[b] -78 °C, THF, lithium base

^[c] All reactions performed in anhydrous conditions under an inert atmosphere.

Table 2

Stille/Suzuki Coupling to form biaryl compounds

Entry ^[a]	Halide	Reagent 2 ^[b]	Product ^[c]	Yield
1	4b	11a	12a	12% ^[a]
2	9a	5b		37% ^[b]
3	9a	5c		96% ^[a]
4	9b	5a	12b	44% ^[b]
5	4a	11b		22% ^[c]

^[a] Pd(II)(OAc)₂, CsF, CuI, P(t-Bu)₃^[b] Pd(PPh₃)₄, CsF, CuI, P(t-Bu)₃^[c] Pd(II)Cl₂, CsF, CuI, P(t-Bu)₃