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A Synthetic Methodology Toward Pyrrolo[2,3-*b*]pyridones for GC Base Pair Recognition

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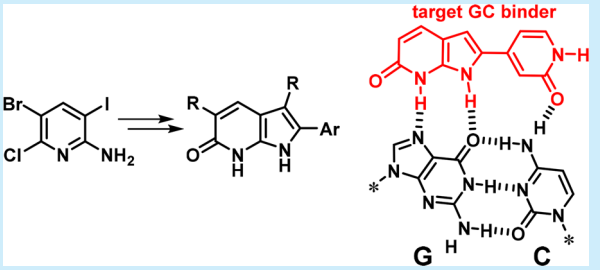
Supporting Information

ABSTRACT: Flexible synthetic access to a novel biarylic GC binding motif is presented, consisting of a pyridone connected to a fused pyrrolo[2,3-*b*]pyridone. Extensive molecular modeling led to an optimized design with perfect complementarity to the Hoogsteen site inside DNA's major groove. A wide range of functional elements can be introduced by minor modifications of the synthetic strategy. Our approach relies on mild Pd-catalyzed coupling reactions, featuring a triple heterohalogenated orthogonally addressable pyridine as a key intermediate.



Nucleic acid recognition is a fundamental biological process and a major prerequisite for gene expression and other important biological events.[1] For sequence-selective binding to be achieved, DNA ligands must target either the major or the minor groove, where the base sequence becomes freely accessible—and readable.[2] Deliberate targeting of specific DNA fragments by synthetic binding agents bears great potential for applications in molecular biology, e.g., the interference with transcription factors and other DNA binding proteins.

The Dervan group has pioneered the sequence-selective recognition of DNA's minor groove by external binders and developed highly promising chemical approaches based on crescent-shaped polyamide derivatives.[3] On the other hand, various classes of artificial nucleic acid derivatives and analogues, such as triplex forming oligonucleotides (TFO)[4] and peptide nucleic acids (PNA),[5] have been invented for the major groove. However, in most cases, recognition is limited to one strand only, prevalently of homopurine nature. In this context, the development of novel nucleosides designed for optimal hydrogen bonding to the natural base pairs within the major groove and improved π-stacking interactions is of great interest for stabilized multiassembly, especially considering the challenging pyrimidine interruptions. A few promising ligands, such as nucleoside S for AT and ureido-biaryl derivatives for GC recognition, have been developed in recent years (Figure 1).[6]

However, a comprehensive system of structurally alike ligands capable of binding selectively to and discriminating between all four possible base pair arrangements has still to be found. In the search for optimal target structures, extensive molecular modeling investigations have been carried out by force field minimizations in our group on a vast number of potential candidates. These finally revealed a pyrrolopyridone-pyridone biarylic ligand as the most promising candidate for GC base pair recognition. GC complexation in silico produces

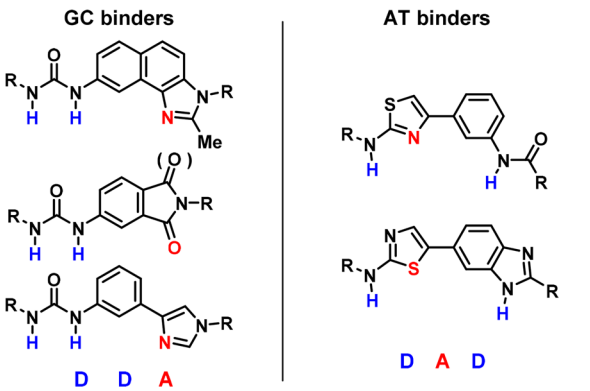


Figure 1. (left) GC base pair binding ureidoaryl derivatives comprising a **DDA** hydrogen-bonding pattern. (right) AT targeting **DAD** ligands S and B_t.[6b−j]

multiple tailored hydrogen bonds and simultaneously addresses both nucleobases in perfect complementarity and remarkable coplanarity to the canonical base pair. Furthermore, its expanded π-system is supposed to contribute to efficient stabilization by favorable stacking interactions between consecutive ligands, as indicated by force field minimizations (Figure 2).

From a medicinal point of view, the fused pyrrolo[2,3-*b*]pyridone system depicted in Figure 2 has been reported in some compounds with biological properties, e.g., p38 kinase inhibitors.[7] This scaffold is also found as a key intermediate in the synthetic route to several drug candidates with anticancer activity reported by Hsieh et al.[8]

We present a new synthesis of the pyrrolopyridone scaffold and its derivatives with various substituents attached to the heterocyclic core, demonstrating its flexibility. For GC

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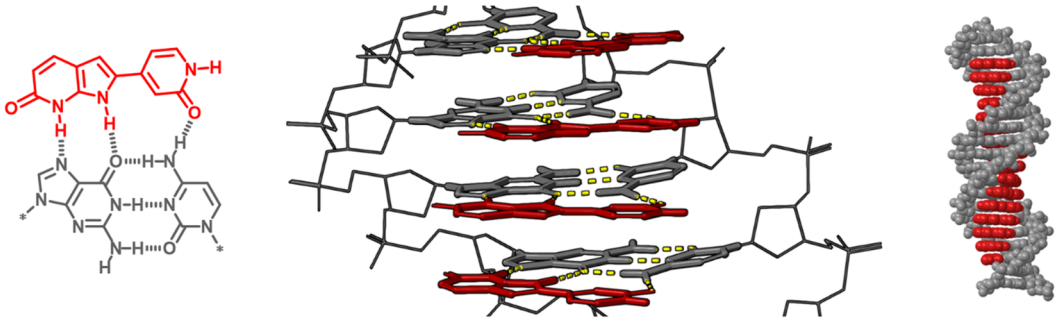
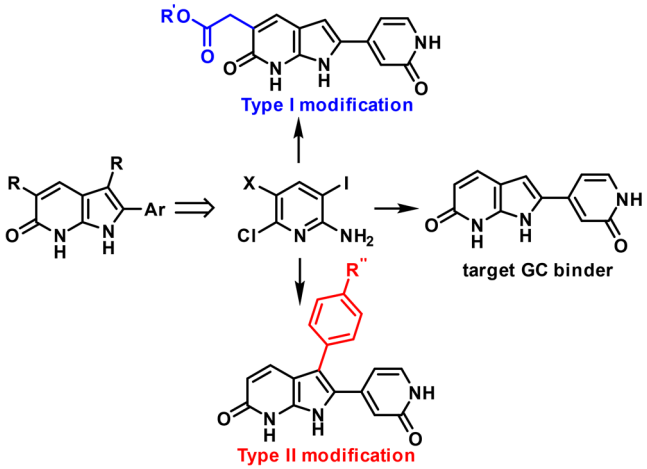


Figure 2. (left) Biarylic pyrrolopyridone-pyridone GC binder forming a base triplet with the GC base pair. (middle) Snapshot from a molecular modeling structure (MacroModel 10.7, OPLS-2005, water, GB/SA) showing four GC binders addressing a set of consecutive GC base pairs via three hydrogen bonds each. (right) Molecular modeling structure of 16 GC binders hydrogen bonded and stacked to a 20 bp GC B-DNA filling up the entire major groove (MacroModel 10.7, OPLS-2005, water, GB/SA).

recognition, a second pyridone unit is placed at the pyrrole-2 position. As the key intermediate, we introduce a di/trihalogenated aminopyridine precursor (X = H, Br) from which all desired derivatives may be prepared (Scheme 1).

Scheme 1. Retrosynthetic Approach for the Preparation of the Parent GC Binder and Its Functional Derivatives Type I and II

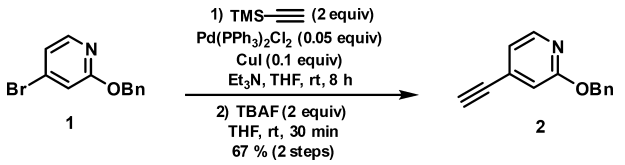


Type I and type II modifications introduce additional functionalities into the GC binder, which are essential for multiple DNA base-pair recognition and improved solubility of model systems in nonpolar solvents. Careful optimization of the entire strategy has produced a set of reactions that offers mild conditions, high functional group tolerance, and ease of purification.

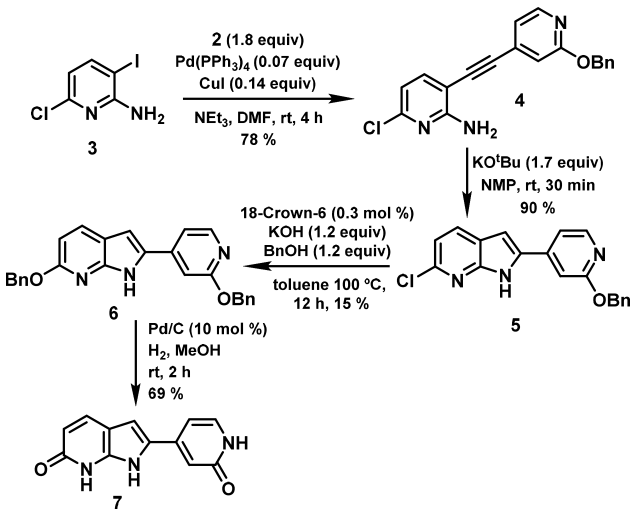
Several strategies for the synthesis of pyridones are available in the literature.[8b,9] Among them, we decided to explore the nucleophilic substitution of 2-chloropyridine with benzyl alcoholate, which may be later reduced with H_2−Pd/C to release the free pyridone. With a Pd-catalyzed Sonogashira cross-coupling reaction in mind as a key step toward the target binder, we first needed to synthesize ethynylpyridine derivative **2**. Starting from known precursor **1**,[10] bromine was displaced in a Sonogashira coupling reaction with trimethylsilylacetylene. Then, the silyl group was removed by TBAF, affording building block **2** (Scheme 2).

Alkyne **2** was subsequently coupled with dihalogenated aminopyridine **3**[11] in another Sonogashira reaction, yielding dipyridylethyne **4** (Scheme 3). For this step, it is essential to

Scheme 2. Synthesis of Ethynylpyridine Derivative 2



Scheme 3. Short Synthetic Route to GC Binder 7



start from an electron-deficient aryl iodide. In previous attempts with a benzoxy group instead of the chloro substituent in **3**, the subsequent Sonogashira coupling reaction did not proceed, most likely because the pyridine ring was too electron-rich for metal insertion. 3-Ethynyl-2-aminopyridine **4**, however, underwent smooth intramolecular cyclization in the presence of base to give pyrrolopyridine **5**. At this stage, chloride was replaced with benzyl alcoholate, and both benzyloxy groups could subsequently be easily removed by catalytic hydrogenation to afford target molecule **7**.

For the purpose of DNA recognition, base pair binder **7** must be modified in such a way that it can be attached to a DNA-compatible backbone with self-repeating units. Importantly, its hydrogen bond forming side must be left unperturbed to maintain base pair complementarity. To this end, we decided to introduce a short C2 spacer at the pyrrolopyridone unit. Type I modification (Scheme 1) includes an ethyl ester substitution at the pyridone-3 position,

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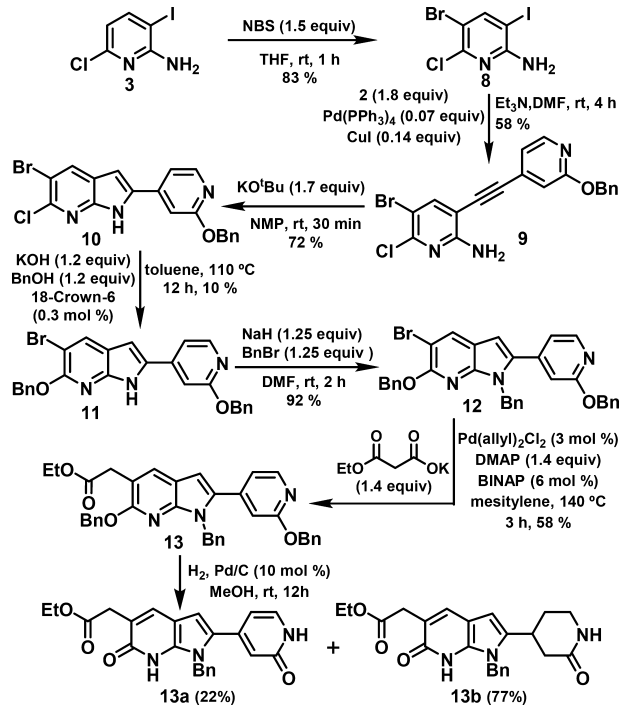
which can later be used as a handle to connect one base pair binder to the next for sequential recognition.

We decided to modify our general strategy outlined in Scheme 3 and added a bromine as third halogen atom to the structure of starting material **3**. It was indeed possible to subject **3** to direct *N*-bromosuccinimide bromination, which gave the desired trihalogen-substituted aminopyridine **8** (Scheme 4). Although there are two positions available for

Scheme 4. Type I Modification of the GC Base Pair Binder

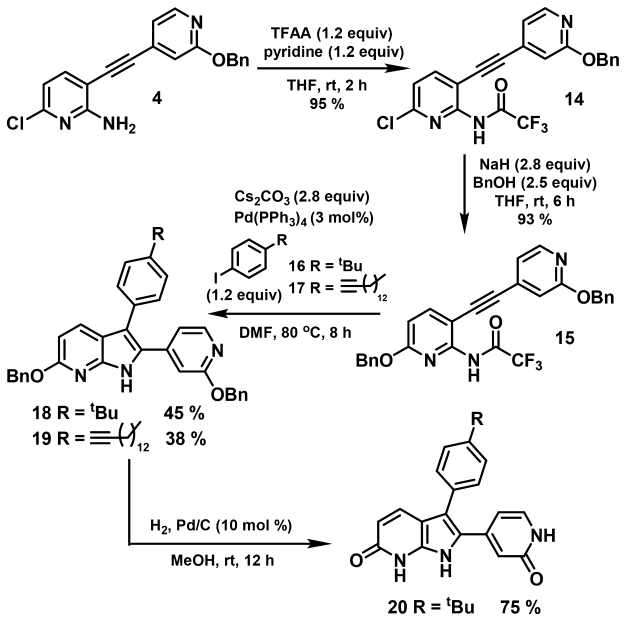


substitution, the bromine atom exclusively occupies the para position with respect to the electron-donating amino group, which is in accordance with the inherent directing effect of the pyridine ring.

Remarkably, the different reactivities of the three halogen atoms could be exploited to address each one selectively in an orthogonal fashion. Thus, iodine as the most reactive leaving group could be selectively substituted during Sonogashira coupling with **2**. The obtained intermediate **9** was again cyclized with base to afford pyrrolopyridine **10**. The chlorine atom ortho to the pyridine nitrogen is most susceptible toward nucleophilic attack and was smoothly substituted by a benzyloxy group to give **11**.[12] Now, the pyrrole NH group was protected as benzylamine to avoid side reactions during cross coupling with the Pd catalyst. The remaining bromine atom was finally displaced in a Pd-catalyzed decarboxylative cross-coupling procedure according to Xu et al.[13] with ethyl potassium malonate to yield **13**. At a later stage, all protecting groups can be removed by debenzylation. At normal pressure, we thus released the two pyridone groups but kept the indole protection.[14]

For the correct hydrogen bonding capability of binder **7** to be established, base triplet formation with an isolated GC base pair must be proven. In the absence of the powerful π-stacking contributions, this must be done in highly nonpolar solvents because polar solvents (even DMSO) will strongly interfere with the three new weak hydrogen bonds. This is a major drawback in the design of all artificial base pair binders; even **7** is not soluble enough in $CDCl_3$ due to the presence of both pyridone amides. Without disturbing the hydrogen bonding recognition site of the binder, we therefore envisaged modifying the back of the pyrrole ring with solubilizing nonpolar substituents (Type II modification, Scheme 1). These derivatives can indeed be conveniently prepared by introducing minor changes in the established synthetic route (Scheme 5). To this end, compound **4** was treated with

Scheme 5. Type II Modification of the GC Binder



trifluoroacetic anhydride to protect the amine in the form of trifluoroacetamide **14**. Chloride was then substituted by benzyl alcoholate to give **15**. Finally, we applied the protocol introduced by Cacchi et al.,[15] which employs $Pd(PPh_3)_4$, Cs_2CO_3, and an aryl halide to effect pyrrole cyclization, insertion of the aryl halide, and deprotection of the trifluoracetamide in one pot and obtained **18** or **19**. These masked GC binders can be debenzylated to release the free corresponding dipyridones. As an example, **18** was smoothly reduced to the corresponding dipyridone **20.**

In summary, we have created a new GC base pair binder based on the rigid pyrrolopyridone scaffold. Its design ensures perfect complementarity to the Hoogsteen site of GC base pairs and allows multiple triplex formation inside DNA's major groove. A versatile synthetic strategy is presented that offers rapid access to various derivatives functionalized at will. It starts from a di- or trihalogenated precursor, whose different halogens can be addressed orthogonally by mild Pd-catalyzed coupling reactions. With a related new AT binder, we intend to create a modular system for sequence-selective DNA recognition in the major groove.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03111.

Experimental procedures and spectroscopic characterization of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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