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Synthesis of DNA-coupled isoquinolones and pyrrolidines by solid phase ytterbium- and silver-mediated imine chemistry†

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DNA-encoded libraries of chemically synthesized compounds are an important small molecule screening technology. The synthesis of encoded compounds in solution is currently restricted to a few DNA-compatible and water-tolerant reactions. Encoded compound synthesis of short DNA-barcodes covalently connected to solid supports benefits from a broad range of choices of organic solvents. Here, we show that this encoded chemistry approach allows for the synthesis of DNA-coupled isoquinolones by an Yb(III)-mediated Castagnoli–Cushman reaction under anhydrous reaction conditions and for the synthesis of highly substituted pyrrolidines by Ag(I)-mediated 1,3-dipolar azomethine ylide cycloaddition. An encoding scheme for these DNA-barcoded compounds based on a DNA hairpin is demonstrated.

Introduction

Endowing chemically synthesized small molecules with barcoded information on their molecular structure **1** is a highly efficient strategy to handle large compound numbers for screening purposes (Fig. 1A). Not the least due to impressive technological advances in the efficiency to read massive genomic data sets, DNA is a highly attractive compound identifier. Genetically tagged collections of small molecules, commonly termed DELs (DNA-encoded libraries), have attracted much attention in recent years as an efficient technology for target-based screening.[1–7] Unlike discrete compound libraries that require cost-intensive infrastructure for screening and biochemical or cell-based assays compatible with high-throughput experimentation,[8] DELs are selected on minute amounts of target proteins either immobilized or captured on a solid support. Thus, through incorporating organic preparative small molecule synthesis for library preparation, as a technology, DELs are much more related to display libraries, such as phages, than to classical small molecule screening collections. Selection of encoded libraries on target proteins has led to the identification of a number of bioactive compounds with unique modes of action such as allosteric GPCR antagonists **2** and **3** and a protein homodimerizer **4** (Fig. 1B).[9–11] Recently, the RIP1 kinase inhibitor **5** evolved from a DEL selection entering clinical

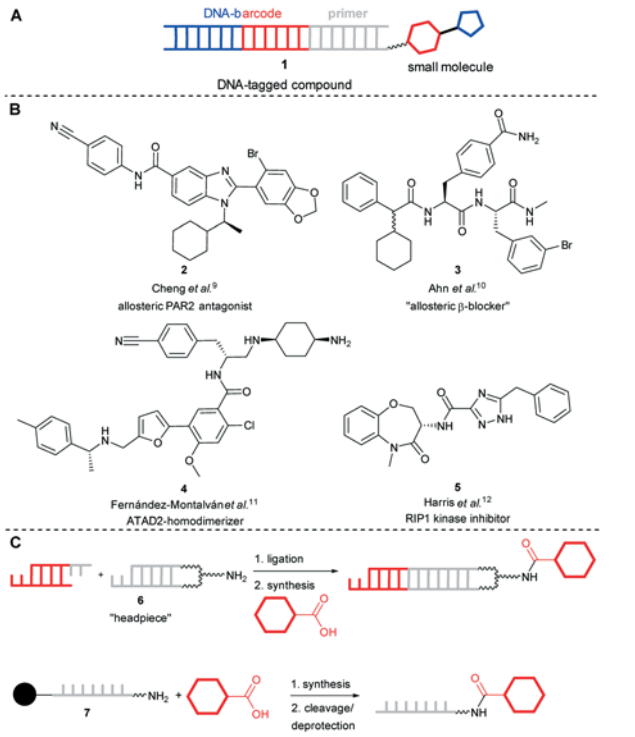

Fig. 1 DNA-encoded library technology. (A) Schematic presentation of a genetically tagged molecule. (B) Exemplary bioactive compounds developed from "hits" identified by DEL selection. (C) Encoded library synthesis may be initiated with a headpiece, **6**, a hairpin-like DNA with a terminal aminolinker for attachment of chemical building blocks; or with a protected single-stranded DNA bound to a solid phase, **7**.

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† Electronic supplementary information (ESI) available: Supplementary data associated with this article can be found in the online version. This includes synthesis and DNA ligation protocols and extended tables for reaction optimization experiments and the reaction scope. Copies of HPLC traces and MALDI-MS spectra are shown for all discussed DNA conjugates. See DOI: 10.1039/c9md00042a

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phase 2, showing the potential of the technology for drug discovery projects.[12] Encoded libraries are synthesized by alternating organic preparative synthesis and DNA ligation steps that track compound synthesis (Fig. 1C). Usually, DEL chemists are planning combinatorial strategies for compound synthesis to access high numbers of molecules efficiently.[13–19]

Library synthesis can for instance be initiated by a hairpin-like "headpiece" structure that contains a linker moiety for attachment of small organic building blocks (Fig. 1C).[14] For this strategy, any synthesis method applied to DEL synthesis needs to be DNA-compatible[20] and it must tolerate water as a (co-)solvent too, requiring *e.g.* development of ligands for metal-mediated reactions.[21–27] Initiating DEL synthesis with a solid support-based synthesis strategy is a viable alternative. The DNA oligonucleotide 7 modified with a linker moiety is synthesized by standard phosphoramidite chemistry on a controlled pore glass (CPG) solid phase. The solid phase material can directly be used for a chemical reaction, *e.g.* coupling of a building block by carbonyl chemistry or nucleophilic substitution reactions for encoded library synthesis or DNA labelling, as demonstrated by different research groups (Fig. 1C).[28–33] In the context of DNA labelling, this methodology is called "post-synthetic" modification and comprised, for instance, Pd-mediated Sonogashira and Stille reactions using standard catalyst–ligand combinations.[34–36] A disadvantage of this strategy is the need to deprotect and cleave oligonucleotide conjugates from the solid phase with concentrated ammonia solution which is an additional step and may damage target molecules by *e.g.* hydrolysis.[37] This disadvantage must be weighed against the benefits of solid support chemistry. These are fully nucleobase-protected DNA which may display higher stability against reagents, and equally important, the choice of a broad range of (dry) organic solvents to perform reactions. Furthermore, the solid phase can be washed extensively to remove excess reactants and reagents such as metal catalysts which might otherwise contaminate the DNA oligomer.

We have a long-standing interest in developing encoding schemes and synthesis methods that give access to genetically tagged heterocycles from simple, readily available starting materials.[38] This research direction in encoded chemistry is justified by the strong representation of heterocyclic structures among natural products and synthetic bioactive compounds including drugs.[39–42] Following up on our systematic investigations in Au(I)-mediated reactions for heterocycle formation on different solid phase-coupled DNA oligonucleotides, among them the chemically highly stable adapter hexT, oligopyrimidine, A/T/C, and A/T/C/G DNA sequences,[38,43] and especially the finding that an A/T/C-sequence tolerated a Au(I)-mediated spirocyclization reaction at room temperature,[43] we decided to investigate further metal-mediated reactions that yield heterocyclic, drug-like structures under mild, ambient reaction conditions. Our focus was placed on imine chemistry. Imines are readily condensed from diverse aldehydes and amines giving rise to high appendage diversity, and they can be cyclized to different heterocyclic structures depending on the addition of further reactants and the mode of catalysis selected. Thus, imine chemistry is an attractive access to structural diversity.[41] However, many imine-based reactions are likely incompatible with aqueous (co)-solvents due to competing hydrolysis of the imine. Here, we show the translation of the $Yb(OTf)_3$-mediated Castagnoli–Cushman reaction into a DNA-tagged format giving rise to diverse substituted tetrahydroquinolones, and the translation of a Ag(I)-mediated [3+2]-cycloaddition yielding highly substituted pyrrolidines. Both reactions were successfully performed on short 10–14mer DNA strands coupled to a CPG solid phase. Following the reaction development, we demonstrate a preliminary scope of both reactions, the limitations of this strategy when applied to longer DNA oligonucleotides and to the acid-mediated Pictet–Spengler reaction, and finally a ligation strategy for short single-stranded 14mer coding strands based on a DNA hairpin sequence.

$Yb(OTf)_3$-mediated Castagnoli–Cushman reaction on CPG-bound oligonucleotides

The Castagnoli–Cushman reaction is a well-established, versatile three component reaction yielding substituted lactams from diverse aldehydes, amines and cyclic anhydrides.[44–48] Among the latter, homophthalic anhydride is particularly frequently used as it furnishes target tetrahydroisoquinolonic acids such as **8–10** under mild conditions.[49–58] This scaffold can be found in numerous bioactive compounds (Fig. 2A). For instance, Floyd *et al.* reacted the acid further with anilines. The target tetrahydroisoquinolone carboxanilides showed potent antimalarial activity and compound **8** was selected as a preclinical candidate.[59,60] Rothweiler *et al.* identified **9** as an inhibitor of the p53-MDM2 interaction.[61,62] Humphries *et al.* characterized tetrahydroisoquinolonic acid **10** as a GPR40 antagonist.[63]

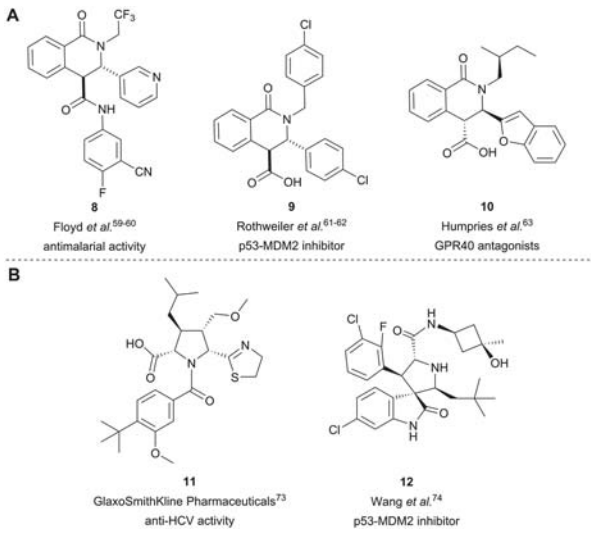


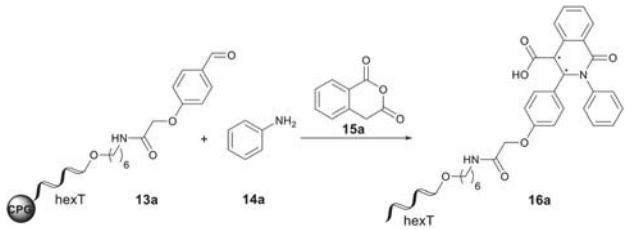
Fig. 2 Representative bioactive tetrahydroisoquinolonic acids (A) and pyrrolidines (B).

Attracted by its demonstrated potential for screening library synthesis, we were highly interested in translating the Castagnoli–Cushman reaction to a solid phase DEL synthesis. The mild reaction conditions reported by Wang *et al.* using $Yb(OTf)_3$ for the Castagnoli–Cushman reaction were selected as a starting point for the reaction translation.[53] We started our investigations with the CPG-bound adapter oligonucleotide "hexT" as previously described.[38] In a first step, we coupled 2-(4-formylphenoxy)acetic acid to the C_6-amino-linker of the hexT. This aldehyde conjugate **13a** was treated in a two-step process first for 4 hours at room temperature with 500 equiv. of aniline **14a** in dichloromethane/triethyl orthoformate to form the imine *in situ*, followed by 100 equiv. of $Yb(OTf)_3$ and 500 equiv. of homophthalic anhydride **15a** (Table 1) that were reacted with the imine for 2 hours at room temperature. Following the reaction, the hexT-conjugates were cleaved from the solid phase and analyzed by analytical HPLC and MALDI-MS. Product conversion was estimated by integration of the product and starting material peaks in the HPLC traces.

However, only traces of the desired product **16a** were observed when the excess of aniline **14a** was reduced by a washing step prior to addition of homophthalic anhydride **15a** (Table 1, entry 1). To our delight, we obtained a 72% conversion into the hexT-lactam conjugate **16a** by direct addition of $Yb(OTf)_3$ and the homophthalic anhydride **15a** suspensions to the *in situ*–formed DNA-imine conjugate, in spite of the high excess of aniline present in the reaction mixture (Table 1, entry 2).

Taking this as the starting point, the reaction conditions were systematically optimized. Decreasing the reaction time for the Castagnoli–Cushman reaction from two hours to one hour had no effect on the product conversion (Table 1, entries 2 and 3). Further decreasing to 0.5 hours led to a 5% lower conversion (Table 1, entry 4). Next, we reduced stepwise the time for the imine formation and observed a slight time-dependent decrease for the conversion too (Table 1, entries 3, 5–6). On the other hand, prolonging the time for imine formation to 16 hours did not improve the yield (Table 1, entry 8). Omitting triethyl orthoformate during imine formation led to a meagre 7% product formation (Table 1, entry 9) showing the need to promote the reaction by this condensing agent. Lowering the $Yb(OTf)_3$ loading from 100 to 50 equiv.

Table 1 Optimization of the Yb(OTf)3-mediated Castagnoli–Cushman reaction to the CPG-bound hexT-isoquinolonic acid conjugate **16a**[a]



Entry	$Yb(OTf)_3$ [equiv.]	t_1 [h]	t_2 [h]	Solvent	Conversion[b] [%]
1[c]	100	4	2	CH_2Cl_2	<5
2	100	4	2	CH_2Cl_2	72
3	100	4	1	CH_2Cl_2	73
4	100	4	0.5	CH_2Cl_2	68
5	100	3	1	CH_2Cl_2	69
6	100	2	1	CH_2Cl_2	68
7	100	1	1	CH_2Cl_2	65
8	100	16	1	CH_2Cl_2	73
9[d]	100	4	1	CH_2Cl_2	7
10	50	4	1	CH_2Cl_2	70
11	25	4	1	CH_2Cl_2	54
12	0	4	1	CH_2Cl_2	38
13[e]	50	4	1	CH_2Cl_2	65
14[f]	50	4	1	CH_2Cl_2	63
15[g]	50	4	1	CH_2Cl_2	51
16	50	4	1	THF	56
17	50	4	1	DCE	58
18	50	4	1	ACN	52
19	50	4	1	MeOH	68
20	50	4	1	Toluene	50
21	50	4	1	1,4-Dioxane	52
22	50	4	1	DMF	31
23	50	4	1	EtOAc	40

[a] CPG-bound oligonucleotide conjugate **13a** (20 nmol) and aniline **14a** (500 equiv., 10 μmol) in 36 μL of solvent/triethyl orthoformate (2 : 1) at ambient temperature for t_1, then $Yb(OTf)_3$ (*X* equiv., 1 μmol) and anhydride **15a** (500 equiv., 10 μmol) both suspended in 30 μL of solvent, at ambient temperature for t_2. Afterwards, with AMA (30% aqueous ammonia/40% aqueous methylamine, 1 : 1 (vol/vol)) at ambient temperature for 0.5 h. [b] Determined by analytical RP-HPLC analysis. [c] Washing of the CPG-bound hexT-conjugate after imine formation. [d] Imine formation performed without triethyl orthoformate. [e] 1000 equiv. of aniline **14a** and anhydride **15** were used. [f] 1500 equiv. of aniline **14a** and anhydride **15a** were used. [g] 2000 equiv. of aniline **14a** and anhydride **15** were used.

