DOI: 10.1002/hlca.201900033

FULL PAPER

HELVETICA chimica acta

Screening of Three Transition Metal-Mediated Reactions Compatible with DNA-Encoded Chemical Libraries

Nicholas Favalli,[a] Gabriele Bassi,[a] Tania Zanetti,[a] Jörg Scheuermann,[a] and Dario Neri*[a]

[a] Institute of Pharmaceutical Sciences, ETH Zürich, CH-8093 Zürich, Switzerland, e-mail: neri@pharma.ethz.ch

The article is dedicated to Prof. Dr. *François Diederich*, on the occasion of his Farewell Symposium

The construction of DNA-encoded chemical libraries (DECLs) crucially relies on the availability of chemical reactions, which are DNA-compatible and which exhibit high conversion rates for a large number of diverse substrates. In this work, we present our optimization and validation procedures for three copper and palladium-catalyzed reactions (*Suzuki* cross-coupling, *Sonogashira* cross-coupling, and copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC)), which have been successfully used by our group for the construction of large encoded libraries.

Keywords: DNA-encoded chemical libraries, *Suzuki* coupling, *Sonogashira* coupling, CuAAC reaction, copper, palladium, DNA-compatible reaction.

Introduction

The encoding of individual small organic molecules with distinctive DNA fragments, serving as amplifiable identification barcodes, allows the construction and screening of chemical libraries of unprecedented size.[1–11] Libraries containing billions of compounds from a relatively small number of building blocks (*e.g.*, few thousand chemical compounds) can be constructed using a variety of different experimental approaches, such as pool-and-split methodologies,[12] DNA-templated chemical reactions with preformed oligonucleotide derivatives,[13–15] or the use of encoded self-assembling strategies.[16–21] The attractiveness of encoding chemical compounds with DNA fragments relate to the possibility of constructing and storing large libraries as a mixture, that can be interrogated by affinity capture procedures on immobilized proteins of interest, followed by decoding, using high-throughput DNA sequencing.[12,22,23] Indeed, the identity and relative frequency of each compound in the library (*e.g.*, before and after a screening experiment) can be directly retrieved from the results of a DNA sequencing experiment, since each library member is encoded by a distinctive DNA fragment.[12,22,23] The construction of large DNA-encoded chemical libraries crucially relies on the availability of 'robust' reactions, which can be performed in water, are DNA-compatible,[24–26] and which accept a large number of structurally diverse substrates. One of the most widely used reactions for the construction of DNA-encoded chemical libraries is the formation of amide bonds, since excellent procedures are available,[24,27] and since thousands of building blocks (*e.g.*, amines and carboxylic acids) can be purchased from commercial sources at moderate costs. However, even a robust reaction such as amide bond formation with DMT-MM methodology displays acceptable conversion yields only for approximately 44% of carboxylic acids. For these reasons, improved methodologies are constantly being explored.[27]

In this work, we aimed at implementing and optimizing copper and palladium-catalyzed reactions, which could be readily used for the construction of DNA-encoded chemical libraries.[28–31] We focused on *Suzuki* cross-coupling, *Sonogashira* cross-coupling, and copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC). There have been recent investigations on the

Supporting information for this article is available on the WWW under https://doi.org/10.1002/hlca.201900033

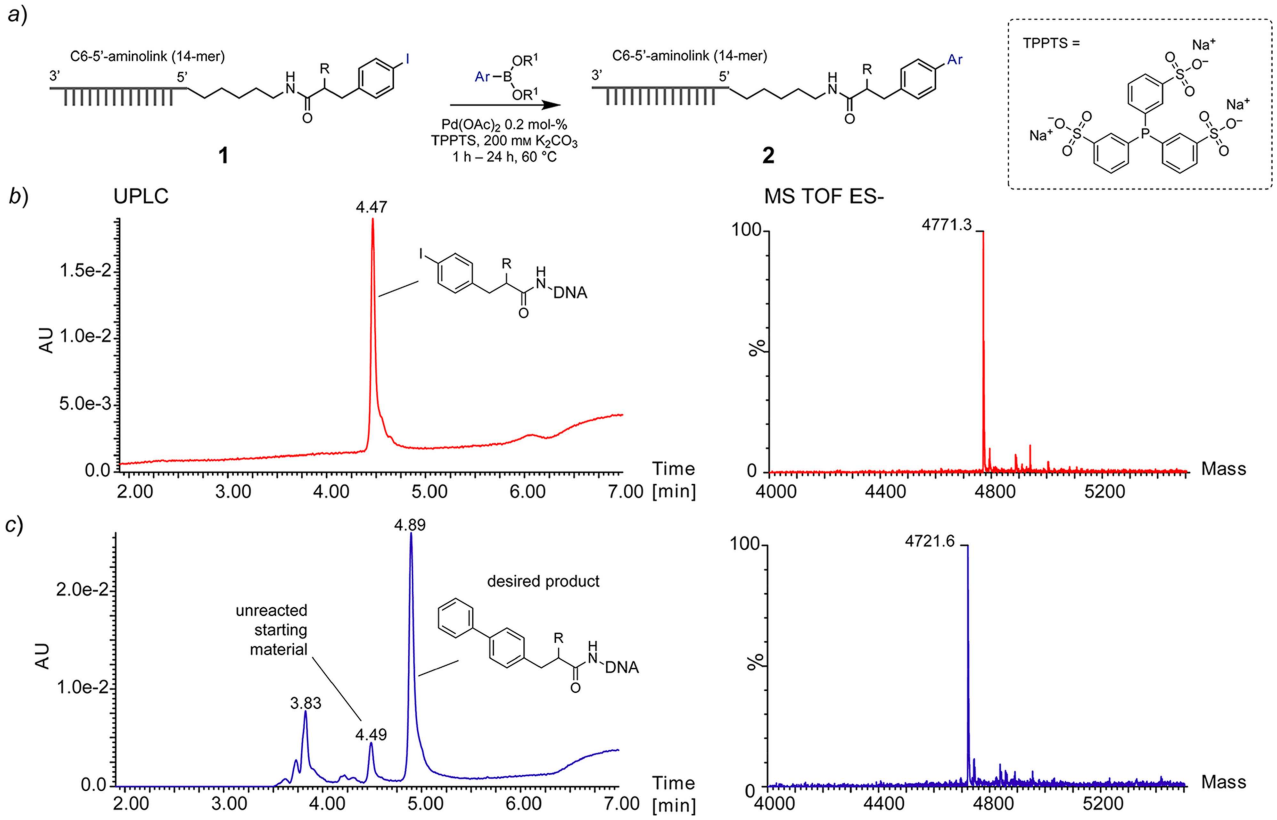


Figure 1. *a*) Palladium-catalyzed *Suzuki* cross-coupling reactions between aryl halides (iodide and bromide) - DNA conjugates and boronic acids or esters. *b*) UPLC chromatograms registered at 260 nm of starting material (**1**) and the deconvoluted ESI-TOF mass spectrum of the corresponding peak (4.47). *c*) Chromatogram of crude *Suzuki* reaction between **1** and phenylboronic acid (**B3**) and the deconvolution of ESI-TOF mass spectrum of the product (4.89); R = N_3, 2-azido-3-(4-iodophenyl)propanamide; R^1 = H, pinacol ester. Ar: all structures of the tested boronic acid were reported in the *Supporting Information, Table S1*.

implementation of these reactions on substrates coupled to double-stranded DNA fragments.[24,32–40] However, it is often convenient to construct libraries using single-stranded DNA fragments, as these molecular assemblies can be expanded using encoding self-assembling chemistry (ESAC)[16–21] or used for innovative screening techniques (*e.g.*, interaction-dependent PCR[41] or the hybridization with complementary oligonucleotide-photocrosslinker conjugates.[42,43] In principle, the DNA-compatibility of a reaction could be different for single- and double-stranded DNA derivatives, due to the different shielding of the bases.

Results and Discussion

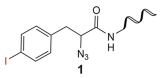
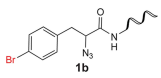
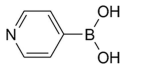
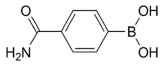
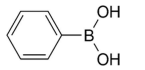
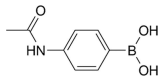
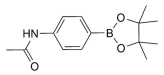
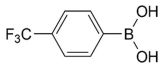
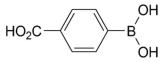
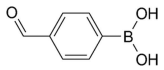
Suzuki *Cross-Coupling*

We first focused on *Suzuki* coupling procedures, since these transformations are broadly used in Medicinal Chemical research for the formation of C–C bonds.[44] *Figure 1* illustrates the reaction of a *p*-iodophenyl derivative, coupled to an amino-tagged single-stranded oligonucleotide comprising 14 bases, with phenyl boronic acid. The purity and identity of the DNA conjugates is typically monitored by UPLC/MS, thus allowing an experimental determination of the performance of the reaction. Throughout the manuscript, this methodology has been used for the calculation of conversion yields and for the characterization of optimal reaction conditions.

As a first step, we explored conversion yields for the reaction of various boronic acids with iodophenyl or bromophenyl propionic acid derivatives on single-stranded DNA, using $Pd(OAc)_2$ as catalyst (*Table 1*). Overall, conversion yields were better for the iodophenyl derivative, which convinced us to focus on iodinated aromatic compounds for further investigations.

We then explored different sources and equivalents of palladium complexes, as well as temperature

Table 1. *Suzuki* cross-coupling performed on aryl iodide and aryl bromide DNA conjugates. The reactions were carried out at 60 °C for three hours using 0.4 equiv. of $Pd(OAc)_2$ – TPPTS complex as catalyst. The reported conversions were calculated by integrating UPLC chromatograms of crude reactions registered at 260 nm.

Reactants *vs.* Substrate	 1	 1b
	0%	0%
	88%	0%
	>98%	60%
	>98%	40%
	>98%	40%
	>98%	50%
	70%	0%
	70%	0%

conditions (*Table 2*). We obtained the best results using $Pd(OAc)_2$, using at least 0.4 equivalents of palladium complexes. Reactions were often satisfactory with as little as 0.2 equivalents. The use of >0.4 equivalents did not result in further improvements. The optimal reaction condition was tested on 32 boronic acids (*Figure 2* and *Supporting Information, Table S1*). In 72% of the cases, conversion yields were >75%, while 6% of the boronic acids exhibited conversion yields between 40 and 75%. Importantly, 22% of the substrates exhibited conversions lower than 40%. These boronic acids were typically aliphatic boronates, acrylboronates, *p*-pyridinylboronic acid derivatives, and compounds which are not soluble in DMA/water. Indeed, methyl ester derivatives were deprotected yielding the corresponding carboxylic acids (*Supporting Information, Table S1*).

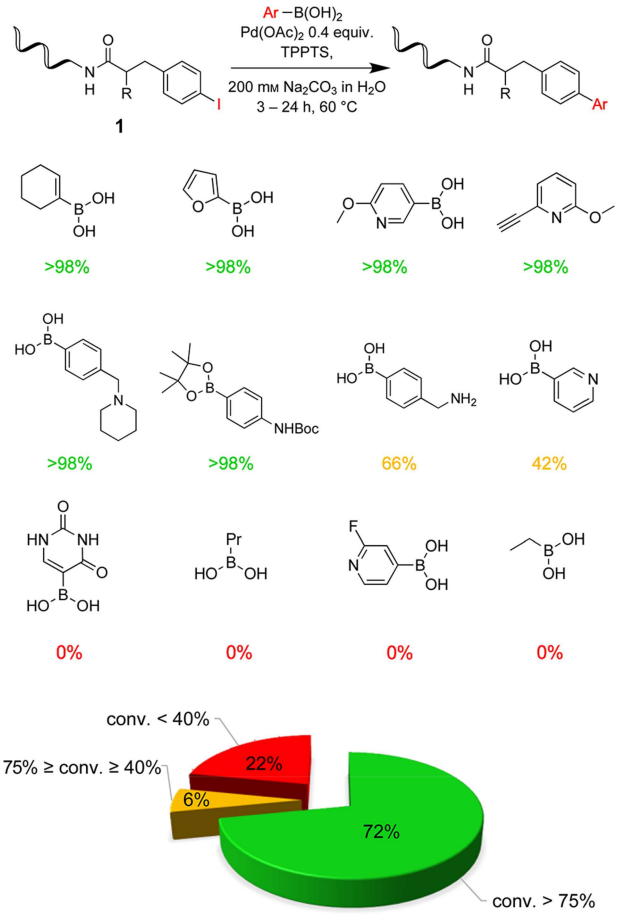
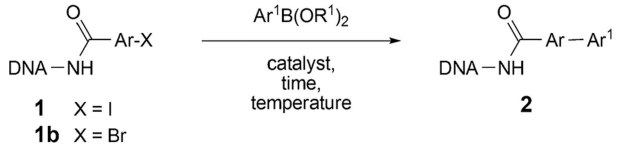


Figure 2. Screening results of *Suzuki* cross coupling reactions between 32 boronic acids and a modified 14-mer oligonucleotide with a *p*-iodo-phenylalanine scaffold. Red, yellow, and green colors correspond to reactions with poor, intermediate, or good conversions. R: N_3, 2-azido-3-(4-iodophenyl)propanamide. Ar: all structures of the tested boronic acid were reported in *Supporting Information, Table S1*.

Table 2. Screening of different catalysts, substrates, and reaction conditions for Suzuki 'on-DNA' cross-couplings. Ar: all structures of the tested boronic acid were reported in *Supporting Information, Table S1*. The test reactions were carried out on an amino-modified oligonucleotide coupled to 2-azido-3-(4-iodophenyl)propanoic acid (X=I) or 2-azido-3-(4-bromophenyl)propanoic acid (X=Br).



1 X = I
1b X = Br
2

Pd(II) Source	Time	Temp.	Aryl halides	Equiv. of Pd[a]
$Pd(OAc)_2$	10 min	50 °C	Ar–I	0.2
$PdCl_2$	1 h	60 °C	Ar–Br	0.4
$PdCl_2(PPh_3)_2$	2 h	65 °C		1.0
$[PdCl(allyl)]_2$	4 h	70 °C		1.5
	24 h			2.0
				4.0

[a] Equivalents with respect to DNA–aryl halide conjugate (**1**).

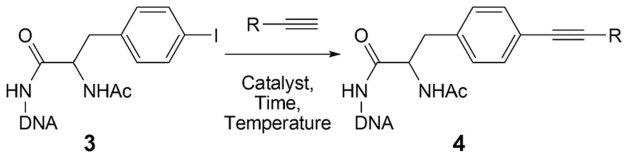
In conclusion, we found that the *Suzuki* reaction could reliably be implemented with iodophenyl DNA

derivatives, using $Pd(OAc)_2$ as catalyst (0.4 equiv., 60 °C, 3–24 h) with aromatic, heteroaromatic, and vinylboronic acids.

Sonogashira *Cross-Coupling*

In full analogy to the optimization of *Suzuki* reaction, we first studied the choice of catalyst (type and equivalents) and of temperature conditions (*Table 3*).

Table 3. Screening of different catalysts, substrates, and reaction conditions for *Sonogashira* 'on-DNA' cross-couplings. R: all structures of the tested alkynes are reported in *Supporting Information, Table S2*. The test reactions were carried out on an amino-modified oligonucleotide coupled to 2-acetamido-3-(4-iodophenyl)propanoic acid.



Pd(II) Source	Cu(II) Source	Time	Temp.	Equiv. of Pd[a]
$Pd(OAc)_2$	$Cu(OAc)_2$	10 min	50 °C	0.2
$PdCl_2$	$Cu(SO_4)_2$	1 h	60 °C	0.5
$PdCl_2(PPh_3)_2$	$Cu(OTf)_2$	2 h	65 °C	1.0
$[PdCl(allyl)]_2$	$Cu(OAc)_2L$	4 h	70 °C	1.5
	Copper-free	24 h		2.0
				4.0

[a] Equivalents with respect to DNA-aryl halide conjugate (**3**).

Various palladium and copper complexes were tested, alone and in combination. The best conversions were obtained for a copper-free reaction using one equivalent of $[PdCl(allyl)]_2$ at 70 °C for three hours. We have observed that the presence of copper at 60 and 70 °C may cleave and degrade the DNA products. An example of *Sonogashira* cross coupling performed with the described conditions was reported in *Figure 3*. The reaction was carried out on a single-strand DNA derivative (compound **3**) with alkyne **A34**. The LC–MS traces show an excellent conversion and side products were not observed.

Figure 4 shows representative coupling results for some of the 44 alkynes that were tested. For 43% of the reagents, >75% conversion was achieved. A conversion between 40% and 75% was observed for 30% of the alkynes, whereas 25% of the compounds exhibited a conversion <40%. 'Difficult' alkynes included structures that were more prone to decomposition. These alkynes were typically amines (A102, A33, A118, A119, A93, A23, and A95), heteroaromatic

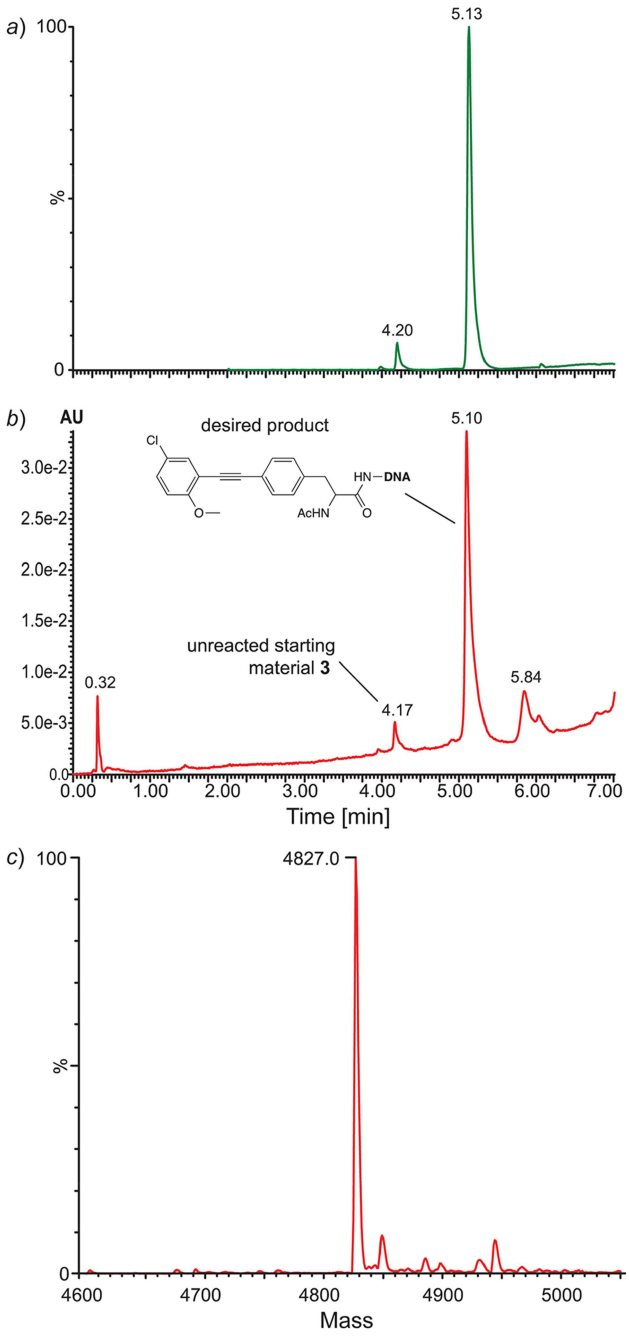


Figure 3. Palladium-catalyzed *Sonogashira* cross-coupling reaction between aryl iodide **3** and 4-chloro-2-ethynyl-1-methoxybenzene (**A34**). *a*) TOF MS spectra and *b*) UPLC chromatogram registered at 260 nm of the crude reaction. *c*) Deconvolution of MS peak of the product (5.13).

aryl-bromides (A6) and compounds with poor water-solubility (A3, A36, and A64). Aromatic aryl-bromides (A32) and aromatic aryl-chlorides (A62, A18, and A34) exhibit excellent conversions. Side products of over-

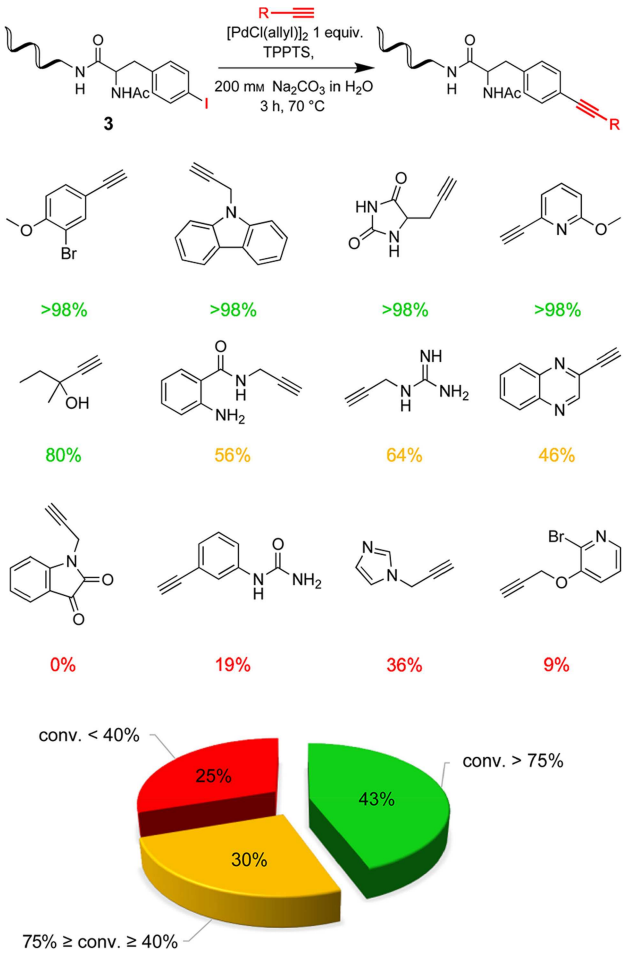


Figure 4. Screening results of *Sonogashira* cross coupling reactions between 44 alkynes and a modified 14-mer oligonucleotide with a *p*-iodo-phenyl moiety. Red, yellow, and green colors correspond to reactions with poor, intermediate, or good conversions. R: all structures of the tested alkynes were reported in *Supporting Information, Table S2*.

reaction were not observed (*Figures 2* and *4* and *Supporting Information, Table S2*).

Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC)

CuAAC is a frequently used reaction for the construction of DNA-encoded chemical libraries,[37,45,46] because of its robustness and of the broad availability of azides and terminal alkynes.

Previous activities of our laboratory featured the use of *pseudo*-solid phase 'on-DNA CuAAC' chemistry. The reactions were performed using organic solvents (DMSO and ^{t}BuOH mixtures) and the conventional TBTA (1-(1-benzyltriazol-4-yl)-*N*,*N*-bis[(1-benzyltriazol-4-yl)methyl]methanamine) as copper (I) ligand.[47]

In order to perform reactions in aqueous solution, with a considerable saving in the amounts of reagents to be used, we synthesized a water-soluble pre-catalyst obtained by mixing copper (II) acetate with a potassium salt of **6** (potassium 4,4′,4′′-[nitrilotris (methylene-1*H*-1,2,3-triazole-4,1-diylmethylene)]tribenzoate). The complex was subsequently reduced *in situ* by sodium ascorbate obtaining the Cu(I) catalyst which promoted efficient conversions, with a broad scope of substrates (*Figure 5*, *Table 4*). The importance of keeping Cu(I) in stable complexes has previously been recognized,[48] as Cu(I) ions may promote DNA cleavage.[48–50]

Table 4. A comparison between pseudo-solid phase CuAAC and the optimized water-compatible CuAAC conditions. The test reactions were carried out on an amino-modified oligonucleotide coupled to 2-azido-3-(4-iodophenyl)propanoic acid.

Reaction Conditions	Pseudo-solid phase CuAAC	Solution-phase CuAAC
Pre-catalyst	$CuSO_4$ + TBTA	$Cu(OAc)_2$ + **6**
Reducing agent	sodium ascorbate	sodium ascorbate
Equiv. of Cu[a]	> 10	0.1
Solvent(s)	DMSO:tBuOH:H_2O mixtures	H_2O
Base	triethylamine	K_2CO_3
Temperature	r.t.	35 °C
Equiv. of Alkyne[a]	400	40
Reaction time	24 h	3 h

[a] Equivalents with respect to azido-DNA conjugate (**1**).

Figure 6 shows representative results for the reaction of an azide derivative on single-stranded DNA with 115 different terminal alkynes. For 65% of the reagents, a conversion > 75% was observed. Only 9% of the alkynes exhibited a conversion between 40% and 75%, while 29% of the reagents showed a worse performance (*Figure 6*, *Supporting Information, Table S3*). Conversions lower than 40% were often observed for hydrophobic compounds which do not readily dissolve in water and for structures (*e.g.*, methyl esters or carboxylic acids).

Conclusions

Suzuki coupling, *Sonogashira* coupling, and copper-catalyzed azide-alkyne cycloaddition reactions were extensively tested with *ca.* 200 substrates. We estab-

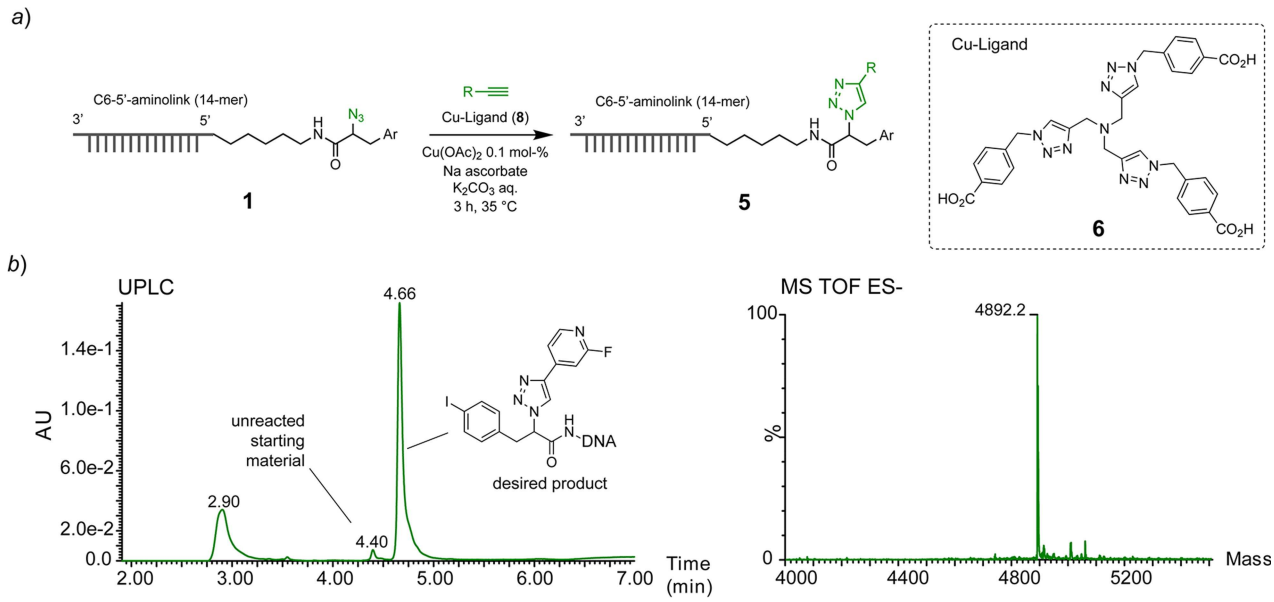


Figure 5. *a*) Copper-catalyzed azido-alkyne cycloaddition (CuAAC) reactions between an azido modified oligonucleotide and alkynes. *b*) UPLC chromatogram of crude CuAAC reaction between **1** and 4-ethynyl-2-fluoropyridine and the deconvolution of ESI-TOF MS spectra of the product (4.66). UPLC chromatogram and MS spectra of starting material **1** is reported in *Figure 1b*. Ar: 4-Iodophenyl; R: all structures of the tested alkynes were reported in *Supporting Information, Table S1*.

lished conditions for all three reactions, which worked well with the majority of the building blocks.

Similar to previous studies with amide bond forming reactions,[27] it is extremely difficult to identify experimental procedures, which yield conversions $>75\,\%$ for all possible substrates.

The construction of DNA-encoded chemical libraries can often afford to be performed using lower conversion rates (*e.g.*, $>40\,\%$), since bioactive compounds are readily identified in screening procedures also when products are not completely pure.[12,15,51–53] Nonetheless, scientists aim at constantly improve library purity, in order to have consistent correlations between enrichment factors in screening experiments and affinity measurements at the hit validation stage.[46,54]

The pie charts presented in the article, describing the cumulative results of conversion rates for *Suzuki* coupling, *Sonogashira* coupling, and copper-catalyzed azide-alkyne cycloaddition reactions, provide a strong rationale for the quantitative evaluation of model reactions prior to library construction. Building blocks which show poor conversions may be replaced by other molecules, which give better yields. The data contained in *Supporting Information, Tables S1–S3* may serve as reference data base for other groups, who may wish to use the same experimental conditions for library construction.

It has often been assumed that double-stranded DNA may be more suitable for library construction, as the heteroduplex could potentially contribute to the integrity of the DNA bases. In our experience, however, single-stranded DNA structures preserve their integrity in the reactions described in this manuscript and in other reactions (data not shown). It is particularly convenient to use single-stranded DNA for library construction, since *n* oligonucleotides are required for the encoding of *n* building blocks. The use of double-stranded DNA fragments typically requires the use of twice the number of oligonucleotides, as well as the need for a linker that bridges and stabilizes the heteroduplex.[13] Single-stranded DNA libraries are conveniently encoded using splint ligation procedures, with short oligonucleotides that can easily be removed at the end of the encoding step.[17,45]

Single-stranded DNA-encoded chemical libraries containing millions of compounds can be easily synthesized.[37,45,46,55] They can be converted into double-stranded DNA using *Klenow* polymerization procedures.[12,45,46,52,54] Alternatively, the pairing of two single-stranded DNA-encoded chemical libraries leads to ESAC libraries of very large size.[16,17,19–21] The total number of compound combinations in ESAC libraries corresponds to the product of the sizes of the individual libraries.

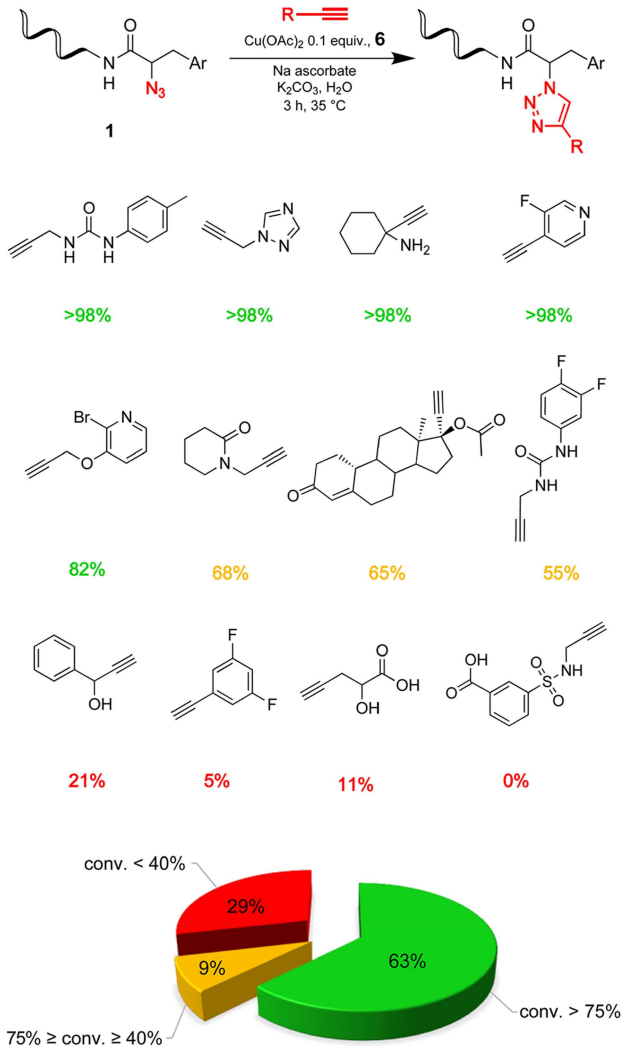


Figure 6. Screening results of CuAAC coupling reactions between 116 alkynes and a modified 14-mer oligonucleotide with a phenylalanine based-scaffold bearing an azido group. Red, yellow, and green colors correspond to reactions with poor, intermediate, or good conversions. Ar: 4-Iodophenyl; R: all structures of the tested alkynes are reported in *Supporting Information, Table S3.*

While efficient reaction conditions could be found for *Suzuki* coupling, *Sonogashira* coupling, and copper-catalyzed alkyne-azide cycloadditions, the field of DNA-encoded chemistry will crucially rely on similar studies on other reactions, in order to expand the chemical diversity that can be used for library screens. While dozens of DNA-compatible reactions have been proposed,[24] the scope of those reactions with a sufficiently broad set of building blocks is often missing.

Experimental Section

General Experimental Information

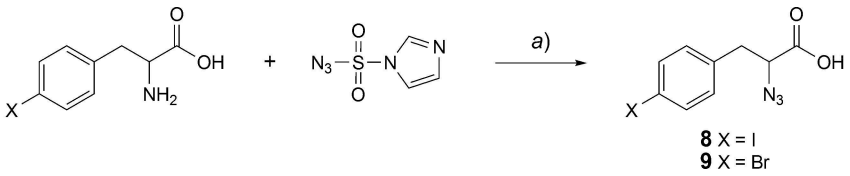
All reagents and solvents used were of analytical grade. Buffers were prepared with ultrapure water (*Merck Millipore Quantum® TEX*). All chemicals were purchased from *Acros*, *Alfa Aesar*, *Sigma-Aldrich*, or *TCI*. The 5′-aminomodified-14mer oligonucleotide (5′-C6-amino-GGAGCTTCTGAATT, MS = 4473 Da) used for tests was purchased by *LGC Biosearch Technologies*. All boronic acid solutions were purchased from *Apollo Scientific* and alkynes solutions were purchased from *Enamine*. Purifications were carried out on a semi-preparative HPLC with *Waters XTerra® Shield RP18* (125 Å, 5 μm) using 100 mM triethylammonium acetate (TEAA pH = 7.0)/acetonitrile (MeCN) as mobile phase (flow = 4.0 mL/min). Databases (*Supporting Information, Table S1–S3*) were managed with Instantjchem 18.

Synthesis of **1** *and* **1b** (*General Procedure* for 'on-DNA' amide coupling formation; *Procedure 1*)

200 mM 2-azido-3-(4-iodophenyl)propanoic acid (**8**; for the synthesis of **1**) and 2-azido-3-(4-bromophenyl) propanoic acid (**9**; for the synthesis of **1b**) were activated by adding 100 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in DMSO (0.95 equiv.) and 100 mM *N*-hydroxysuccinimide (S-NHS) in DMSO/water 2:1 (1.5 equiv.). The resulting mixtures were agitated for 20 min at 30 °C. The 5′-aminomodified oligonucleotide (5′-C6-amino-GGAGCTTCTGAATT, MS = 4473 Da, 1–500 nmol, 1 mM) in 50 mM TEA·HCl buffer (pH = 10) was subsequently added to 100 equiv. of the corresponding activated S-NHS ester. The reactions were heated at 37 °C for 12 h, and the products were precipitated with ethanol and purified by HPLC (*Supporting Information, Figure S1*).

Synthesis of **2** (optimized procedure for 'on-DNA' *Suzuki* cross-coupling)

The catalyst solution was prepared by mixing 20 μL of 10 mM palladium (II) acetate in *N*,*N*-dimethylacetamide (DMA), 100 μL of 100 mM trisodium 3,3′,3-phosphinetriyltribenzenesulfonate (TPPTS) in water and 480 μL of water, resulting in a 0.33 mM solution of Pd(0)-TPPTS complex. To a 0.1 mM solution of oligonucleotide **1** (2 nmol, 20 μL) in water were subsequently added 60 μL of 200 mM Na_2CO_3 in water, 2.5 μL of catalyst solution (0.8 nmol in Pd) and 10 μL of 200 mM ArB



Scheme 1. Synthesis of **8** and **9**. *a*) $CuSO_4$ 2%, K_2CO_3, MeOH, 24 h, r.t.

$(OH)_2$ (*Supporting Information, Table S1*) in DMA. The resulting mixture was agitated from 3 to 24 h at 60 °C.

Synthesis of **3**

The azido oligonucleotide conjugate **1** (100 nmol) was treated with 0.5 mL of 30 mM tris(2-carboxyethyl) phosphine (TCEP) in 500 mM *Tris*·HCl buffer (pH = 7.4) for 3 h at 35 °C yielding the corresponding primary amine **7** (*Supporting Information, Figure S1*). The obtained product was acetylated by adding activated acetic acid S-NHS ester (*Procedure 1*). The product was precipitated and purified by HPLC (*Supporting Information, Figure S1*).

Synthesis of **4** (optimized procedure for 'on-DNA' *Sonogashira* cross coupling)

The catalyst solution was prepared by mixing 100 μL of 10 mM allylpalladium (II) chloride dimer in DMA, 100 μL of 100 mM TPPTS in water and 800 μL of water, resulting in a 1 mM solution of Pd(0)-TPPTS complex. To a 0.1 mM solution of oligonucleotide **3** (2 nmol, 20 μL) in water were subsequently added 50 μL of 200 mM Na_2CO_3 in water, 2 μL of catalyst solution (2 nmol in Pd) and 5 μL of 100 mM alkyne (*Supporting Information, Table S2*) in DMSO. The resulting mixture was agitated for 3 h at 70 °C.

Synthesis of **5** (optimized procedure for 'on-DNA' CuAAC)

All solvents were degassed in argon atmosphere. The pre-catalyst solution was prepared by mixing 25 μL of 10 mM $Cu(OAc)_2$, 100 μL of 10 mM solution of **8** in 200 mM K_2CO_3 and 2'325 μL of water, resulting in a 100 μM solution of Cu(II)-**8** complex. To a 100 mM solution of oligonucleotide **1** (10 nmol in 100 μL) in water were subsequently added 0.1 μL of 50 mM K_2CO_3, 10 μL of pre-catalyst solution (1 nmol) and 40 μL of 10 mM alkyne (*Supporting Information, Table S3*) in DMSO. The solution was mixed and the catalyst was activated by adding 10 μL of 10 mM sodium-L-ascorbate in water. The mixture was agitated at 35 °C for 3 h.

Synthesis of Compounds **8** *and* **9** (*Scheme 1*)

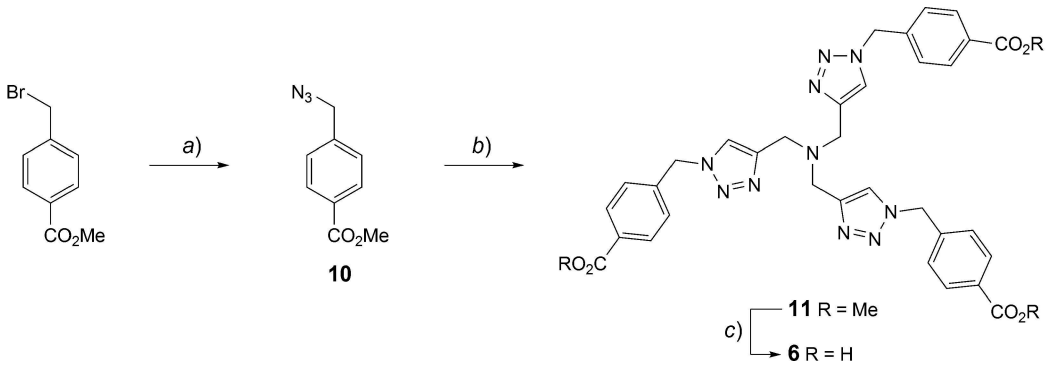
The commercially available *p*-I- or *p*-Br-(D,L)-phenylalanine (5.4 mmol) was dissolved in dry methanol (15 mL) and 1*H*-imidazole-1-sulfonyl azide hydrochloride (1.36 g, 6.5 mmol), anhydrous potassium carbonate (1.86 g, 13.5 mmol), and anhydrous copper sulfate (40 mg, 0.02 mmol) were added, and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated under reduced pressure, and the products were extracted with AcOEt. The pure compounds **8** and **9** (*Scheme 1*) were obtained by silica gel chromatography with hexane/AcOEt 7:3 as an eluent with 56% of yield.

Data of Compound **8**. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.71–7.63 (*m*, 2 H); 7.13–7.04 (*m*, 2 H); 4.41 (*dd*, *J* = 8.7, 5.0, 1 H); 3.06 (*dd*, *J* = 14.2, 5.0, 1 H); 2.89 (*dd*, *J* = 14.2, 8.7, 1 H).

Data of Compound **9**. ^{1}H-NMR (400 MHz, (D_6)DMSO): 7.53–7.42 (*m*, 2 H); 7.26–7.18 (*m*, 2 H); 4.36 (*dd*, *J* = 8.1, 4.6, 1 H); 3.09 (*dd*, *J* = 14.2, 4.6, 1 H); 2.84 (*dd*, *J* = 14.2, 8.1, 1 H).

Synthesis of Cu(I) Ligand **6**

Synthesis of **10**. The commercially available methyl 4-(bromomethyl)benzoate (3.0 g, 13.1 mmol) was dissolved in DMF (20 mL) and then NaN_3 (8.5 g, 130.8 mmol) and water (2 mL) were added (*Scheme 2*). The resulting mixture was heated at 80 °C for 4 h and then concentrated under reduced pressure. The crude product was diluted with CH_2Cl_2 and washed with water. The organic phases were collected and concentrated. The pure product **10** was obtained by silica gel chromatography with hexane/AcOEt 8:2 as an eluent with 96% of yield. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.09–7.99 (*m*, 2 H); 7.40–7.34 (*m*, 2 H); 4.39 (*s*, 2 H); 3.90 (*s*, 3



Scheme 2. Synthesis of **6**. *a*) NaN_3 (10 equiv.), DMF/H_2O 9:1, 80 °C, 4 h. *b*) tripropargylamine, CuI (2%), TBTA (2%), DMF, TEA, r.t., 48 h. *c*) 3 M LiOH, H_2O/MeOH 1:1, 80 °C, 1 h.

H). ^{13}C-NMR (101 MHz, $CDCl_3$): 166.59; 140.42; 130.10; 127.91; 119.99; 54.26; 52.18.

Synthesis of **11**. All solvents were degassed in argon atmosphere. Compound **10** (2.4 g, 12.6 mmol) was dissolved in DMF (10 mL) and tripropargylamine (4.1 mmol, 590 μL), CuI (48 mg, 0.25 mmol) and 100 mg of tris(benzyltriazolylmethyl)amine (TBTA, 132 mg, 0.25 mmol) were added. The mixture was stirred at r.t. for 48 h. The solvent was evaporated under reduce pressure, and the pure product **11** was obtained by silica gel chromatography with CH_2Cl_2/MeOH 9:1 as an eluent with >98% of yield. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.17 (*s*, 3 H); 7.93 (*d*, *J* = 8.2, 5 H); 7.37 (*d*, *J* = 8.3, 6 H); 5.70 (*s*, 6 H); 3.83 (*s*, 9 H); 2.54 (*s*, 6 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 166.27; 141.79; 130.05; 129.71; 128.35; 52.66; 40.91. ESI-TOF-MS (pos.): 623 ([*M* − 3 N_2]$^+$), 651 ([*M* − 2 N_2]$^+$), 679 ([*M* − N_2]$^+$), 706 ([*M* + H]$^+$), 707 ([*M* + 2 H]$^+$), 727 ([*M* + Na]$^+$), 1410 (2 *M*$^+$).

Synthesis of **6**. The methyl ester **11** (1.0 g, 1.41 mmol) was dissolved in MeOH (10 mL) and 3 M LiOH water solution (30 mmol, 10 mL) was added. The resulting suspension was stirred at 80 °C for 1 h. The reaction was cooled and quenched with 3 M HCl. The product **6** was extracted with CH_2Cl_2 and purified by RP-HPLC with 0.1% trifluoroacetic acid (TFA) in water/acetonitrile as mobile phase and obtained with 50% yield. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.13 (*s*, 3 H); 7.94–7.87 (*m*, 6 H); 7.38–7.29 (*m*, 6 H); 5.67 (*s*, 6 H); 3.64 (*s*, 6 H). ^{13}C-NMR (101 MHz, (D_6)DMSO): 167.90; 144.21; 140.63; 132.68; 130.09; 127.98; 124.92; 52.80; 47.43. ESI-TOF-MS (neg.): 661.226 [*M*–H]$^-$), 662.220 (*M*$^-$), 1323.356 ([2 *M*–H]$^-$), 1324.356 (2 *M*$^-$), 1986.485 (3 *M*$^-$). ESI-TOF-MS (pos.): 635.230 ([*M*–N_2]$^+$), 663.207 ([*M* + H]$^+$), 664.236 ([*M* + 2 H]$^+$), 665.238 ([*M* + 3 H]$^+$), 1325.370 ([2 *M* + H]$^+$).

Acknowledgements

Financial support from the ETH Zürich, the *Swiss National Science Foundation* (Grants 30030B_163479),*Sinergia* (CRS112_16099), and from the *ERC Advanced Grant 'ZAUBERKUGEL'* (Grant agreement 670603) is gratefully acknowledged. Instant JChem (ChemAxon) was used for structure and data management (http://www.chemaxon.com).

Author Contribution Statement

N. F. designed and performed the experiments, analyzed the data, and wrote the manuscript, *G. B.* and *T. Z.* performed the experiments, analyzed the data, *J. S.* and *D. N.* conceived the project and wrote the manuscript.

References

[1] S. Brenner, R. A. Lerner, 'Encoded combinatorial chemistry', *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5381–5383.
[2] S. Melkko, C. E. Dumelin, J. Scheuermann, D. Neri, 'Lead discovery by DNA-encoded chemical libraries', *Drug Discovery Today* **2007**, *12*, 465–471.
[3] S. J. Wrenn, P. B. Harbury, 'Chemical Evolution as a Tool for Molecular Discovery', *Annu. Rev. Biochem.* **2007**, *76*, 331–349.
[4] M. A. Clark, 'Selecting chemicals: the emerging utility of DNA-encoded libraries', *Curr. Opin. Chem. Biol.* **2010**, *14*, 396–403.

[5] R. E. Kleiner, C. E. Dumelin, D. R. Liu, 'Small-molecule discovery from DNA-encoded chemical libraries', *Chem. Soc. Rev.* **2011**, *40*, 5707 5717.
[6] K. K. Sadhu, N. Winssinger, 'Nucleic Acid-tagged Peptides: Encoding Libraries and Controlling Dimerization and Conformation', *Chimia* **2013**, *67*, 905–909.
[7] A. I. Chan, L. M. McGregor, D. R. Liu, 'Novel selection methods for DNA-encoded chemical libraries', *Curr. Opin. Chem. Biol.* **2015**, *26*, 55–61.
[8] L. H. Yuen, R. M. Franzini, 'Achievements, Challenges, and Opportunities in DNA-Encoded Library Research: An Academic Point of View', *ChemBioChem* **2017**, *18*, 829–836.
[9] B. Shi, Y. Zhou, Y. Huang, J. Zhang, X. Li, 'Recent advances on the encoding and selection methods of DNA-encoded chemical library', *Bioorg. Med. Chem. Lett.* **2017**, *27*, 361–369.
[10] N. Favalli, G. Bassi, J. Scheuermann, D. Neri, 'DNA-encoded chemical libraries–achievements and remaining challenges', *FEBS Lett.* **2018**, *592*, 2168–2180.
[11] D. Neri, R. A. Lerner, 'DNA-Encoded Chemical Libraries: A Selection System Based On Endowing Organic Compounds with Amplifiable Information', *Ann. Rev. Biochem.* **2018**, *87*, 479–502.
[12] L. Mannocci, Y. Zhang, J. Scheuermann, M. Leimbacher, G. De Bellis, E. Rizzi, C. Dumelin, S. Melkko, D. Neri, 'High-throughput sequencing allows the identification of binding molecules isolated from DNA-encoded chemical libraries', *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17670–17675.
[13] Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder, D. R. Liu, 'DNA-Templated Organic Synthesis and Selection of a Library of Macrocycles', *Science* **2004**, *305*, 1601–1605.
[14] X. Li, D. R. Liu, 'DNA-Templated Organic Synthesis: Nature's Strategy for Controlling Chemical Reactivity Applied to Synthetic Molecules', *Angew. Chem. Int. Ed.* **2004**, *43*, 4848–4870.
[15] P. Blakskjaer, T. Heitner, N. J. V. Hansen, 'Fidelity by design: Yoctoreactor and binder trap enrichment for small-molecule DNA-encoded libraries and drug discovery', *Curr. Opin. Chem. Biol.* **2015**, *26*, 62–71.
[16] S. Melkko, J. Scheuermann, C. E. Dumelin, D. Neri, 'Encoded self-assembling chemical libraries', *Nat. Biotechnol.* **2004**, *22*, 568–574.
[17] M. Wichert, N. Krall, W. Decurtins, R. M. Franzini, F. Pretto, P. Schneider, D. Neri, J. Scheuermann, 'Dual-display of small molecules enables the discovery of ligand pairs and facilitates affinity maturation', *Nat. Chem.* **2015**, *7*, 241–249.
[18] N. Winssinger, 'Nucleic Acid-programmed Assemblies: Translating Instruction into Function in Chemical Biology', *Chimia* **2013**, *67*, 340–348.
[19] J. Scheuermann, C. E. Dumelin, S. Melkko, Y. Zhang, L. Mannocci, M. Jaggi, J. Sobek, D. Neri, 'DNA-Encoded Chemical Libraries for the Discovery of MMP-3 Inhibitors', *Bioconjugate Chem.* **2008**, *19*, 778–785.
[20] F. V. Reddavide, W. Lin, S. Lehnert, Y. Zhang, 'DNA-Encoded Dynamic Combinatorial Chemical Libraries', *Angew. Chem. Int. Ed.* **2015**, *54*, 7924–7928.
[21] Y. Zhou, C. Li, J. Peng, L. Xie, L. Meng, Q. Li, J. Zhang, X. D. Li, X. Li, X. Huang, X. Li, 'DNA-Encoded Dynamic Chemical Library and Its Applications in Ligand Discovery', *J. Am. Chem. Soc.* **2018**, *140*, 15859–15867.
[22] F. Buller, M. Steiner, J. Scheuermann, L. Mannocci, I. Nissen, M. Kohler, C. Beisel, D. Neri, 'High-throughput sequencing for the identification of binding molecules from DNA-encoded chemical libraries', *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4188–4192.
[23] Z. Zhu, J. Cuozzo, 'Review Article: High-Throughput Affinity-Based Technologies for Small-Molecule Drug Discovery', *J. Biomol. Screen.* **2009**, *14*, 1157–1164.
[24] A. L. Satz, J. Cai, Y. Chen, R. Goodnow, F. Gruber, A. Kowalczyk, A. Petersen, G. Naderi-Oboodi, L. Orzechowski, Q. Strebel, 'DNA Compatible Multistep Synthesis and Applications to DNA Encoded Libraries', *Bioconjugate Chem.* **2015**, *26*, 1623–1632.
[25] M. L. Malone, B. M. Paegel, 'What is a "DNA-Compatible" Reaction?', *ACS Comb. Sci.* **2016**, *18*, 182–187.
[26] H. Li, Z. Sun, W. Wu, X. Wang, M. Zhang, X. Lu, W. Zhong, D. Dai, 'Inverse-Electron-Demand Diels–Alder Reactions for the Synthesis of Pyridazines on DNA', *Org. Lett.* **2018**, *20*, 7186–7191.
[27] Y. Li, E. Gabriele, F. Samain, N. Favalli, F. Sladojevich, J. Scheuermann, D. Neri, 'Optimized Reaction Conditions for Amide Bond Formation in DNA-Encoded Combinatorial Libraries', *ACS Comb. Sci.* **2016**, *18*, 438–443.
[28] X. Lu, S. E. Roberts, G. J. Franklin, C. P. Davie, 'On-DNA Pd and Cu promoted C–N cross-coupling reactions', *MedChemComm* **2017**, *8*, 1614–1617.
[29] X. Wang, H. Sun, J. Liu, D. Dai, M. Zhang, H. Zhou, W. Zhong, X. Lu, 'Ruthenium-Promoted C–H Activation Reactions between DNA-Conjugated Acrylamide and Aromatic Acids', *Org. Lett.* **2018**, *20*, 4764–4768.
[30] X. Wang, H. Sun, J. Liu, W. Zhong, M. Zhang, H. Zhou, D. Dai, X. Lu, 'Palladium-Promoted DNA-Compatible Heck Reaction', *Org. Lett.* **2019**, *21*, 719–723.
[31] M. K. Škopić, O. Bugain, K. Jung, S. Onstein, S. Brandherm, T. Kalliokoski, A. Brunschweiger, 'Design and synthesis of DNA-encoded libraries based on a benzodiazepine and a pyrazolopyrimidine scaffold', *MedChemComm* **2016**, *7*, 1957–1965.
[32] E. de Pedro Beato, J. Priego, A. Gironda-Martínez, F. González, J. Benavides, J. Blas, M. D. Martín-Ortega, M. A. Toledo, J. Ezquerra, A. Torrado, 'Mild and Efficient Palladium-Mediated C–N Cross-Coupling Reaction between DNA-Conjugated Aryl Bromides and Aromatic Amines', *ACS Comb. Sci.* **2019**, *21*, 69–74.
[33] J.-Y. Li, H. Huang, 'Development of DNA-Compatible Suzuki-Miyaura Reaction in Aqueous Media', *Bioconjugate Chem.* **2018**, *29*, 3841–3846.
[34] A. H. El-Sagheer, T. Brown, 'Click chemistry with DNA', *Chem. Soc. Rev.* **2010**, *39*, 1388–1405.
[35] G. Hervé, C. Len, 'Heck and Sonogashira couplings in aqueous media–application to unprotected nucleosides and nucleotides', *Sustain. Chem. Proc.* **2015**, *3*, 3.
[36] L. Fan, C. P. Davie, 'Zirconium(IV)-Catalyzed Ring Opening of on-DNA Epoxides in Water', *ChemBioChem* **2017**, *18*, 843–847.
[37] A. Litovchick, C. E. Dumelin, S. Habeshian, D. Gikunju, M. A. Guié, P. Centrella, Y. Zhang, E. A. Sigel, J. W. Cuozzo, A. D. Keefe, M. A. Clark, 'Encoded Library Synthesis Using Chemical Ligation and the Discovery of sEH Inhibitors from a 334-Million Member Library', *Sci. Rep.* **2015**, *5*, 10916.

[38] Y. Ding, G. J. Franklin, J. L. DeLorey, P. A. Centrella, S. Mataruse, M. A. Clark, S. R. Skinner, S. Belyanskaya, 'Design and Synthesis of Biaryl DNA-Encoded Libraries', *ACS Comb. Sci.* **2016**, *18*, 625–629.

[39] Y. Ding, M. A. Clark, 'Robust Suzuki-Miyaura Cross-Coupling on DNA-Linked Substrates', *ACS Comb. Sci.* **2014**, *17*, 1–4.

[40] Y. Ding, J. L. DeLorey, M. A. Clark, 'Novel Catalyst System for Suzuki-Miyaura Coupling of Challenging DNA-Linked Aryl Chlorides', *Bioconjugate Chem.* **2016**, *27*, 2597–2600.

[41] L. M. McGregor, D. J. Gorin, C. E. Dumelin, D. R. Liu, 'Interaction-Dependent PCR: Identification of Ligand–Target Pairs from Libraries of Ligands and Libraries of Targets in a Single Solution-Phase Experiment', *J. Am. Chem. Soc.* **2010**, *132*, 15522–15524.

[42] G. Li, Y. Liu, Y. Liu, L. Chen, S. Wu, Y. Liu, X. Li, 'Photoaffinity Labeling of Small-Molecule-Binding Proteins by DNA-Templated Chemistry', *Angew. Chem. Int. Ed.* **2013**, *52*, 9544–9549.

[43] D.-Y. Wang, Y. Cao, L.-Y. Zheng, L.-D. Chen, X.-F. Chen, Z.-Y. Hong, Z.-Y. Zhu, X. Li, Y.-F. Chai, 'Target Identification of Kinase Inhibitor Alisertib (MLN8237) by Using DNA-Programmed Affinity Labeling', *Chem. Eur. J.* **2017**, *23*, 10906–10914.

[44] N. G. Schmidt, E. Eger, W. Kroutil, 'Building Bridges: Biocatalytic C–C-Bond Formation toward Multifunctional Products', *ACS Catal.* **2016**, *6*, 4286–4311.

[45] Y. Li, R. De Luca, S. Cazzamalli, F. Pretto, D. Bajic, J. Scheuermann, D. Neri, 'Versatile protein recognition by the encoded display of multiple chemical elements on a constant macrocyclic scaffold', *Nat. Chem.* **2018**, *10*, 441–448.

[46] N. Favalli, S. Biendl, M. Hartmann, J. Piazzi, F. Sladojevich, S. Gräslund, P. J. Brown, K. Näreoja, H. Schüler, J. Scheuermann, R. Franzini, D. Neri, 'A DNA-Encoded Library of Chemical Compounds Based on Common Scaffolding Structures Reveals the Impact of Ligand Geometry on Protein Recognition', *ChemMedChem* **2018**, *13*, 1303–1307.

[47] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, 'Polytriazoles as Copper(I)-Stabilizing Ligands in Catalysis', *Org. Lett.* **2004**, *6*, 2852–2855.

[48] D.-D. Li, J.-L. Tian, W. Gu, X. Liu, S.-P. Yan, 'A novel 1,2,4-triazole-based copper(II) complex: synthesis, characterization, magnetic property and nuclease activity', *J. Inorg. Biochem.* **2010**, *104*, 171–179.

[49] L. A. Basile, A. L. Raphael, J. K. Barton, 'Metal-Activated Hydrolytic Cleavage of DNA', *J. Am. Chem. Soc.* **1987**, *109*, 7550–7551.

[50] D. C. A. John, K. T. Douglas, 'A common chemical mechanism used for DNA cleavage by copper(II) activated by thiols and ascorbate is distinct from that for copper(II): hydrogen peroxide cleavage', *Transition Met. Chem.* **1996**, *21*, 460–463.

[51] W. Decurtins, M. Wichert, R. M. Franzini, F. Buller, M. A. Stravs, Y. Zhang, D. Neri, J. Scheuermann, 'Automated screening for small organic ligands using DNA-encoded chemical libraries', *Nat. Protoc.* **2016**, *11*, 764–780.

[52] M. Leimbacher, Y. Zhang, L. Mannocci, M. Stravs, T. Geppert, J. Scheuermann, G. Schneider, D. Neri, 'Discovery of Small-Molecule Interleukin-2 Inhibitors from a DNA-Encoded Chemical Library', *Chem. Eur. J.* **2012**, *18*, 7729–7737.

[53] P. Zhao, Z. Chen, Y. Li, D. Sun, Y. Gao, Y. Huang, X. Li, 'Selection of DNA-Encoded Small Molecule Libraries Against Unmodified and Non-Immobilized Protein Targets', *Angew. Chem. Int. Ed.* **2014**, *53*, 10056–10059.

[54] R. M. Franzini, T. Ekblad, N. Zhong, M. Wichert, W. Decurtins, A. Nauer, M. Zimmermann, F. Samain, J. Scheuermann, P. J. Brown, J. Hall, S. Gräslund, H. Schuler, D. Neri, 'Identification of Structure-Activity Relationships from Screening a Structurally Compact DNA-Encoded Chemical Library', *Angew. Chem. Int. Ed.* **2015**, *54*, 3927–3931.

[55] S. L. Belyanskaya, Y. Ding, J. F. Callahan, A. L. Lazaar, D. I. Israel, 'Discovering Drugs with DNA-Encoded Library Technology: From Concept to Clinic with an Inhibitor of Soluble Epoxide Hydrolase', *ChemBioChem* **2017**, *18*, 837–842.

Received January 31, 2019
Accepted March 4, 2019