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Large screening of DNA-compatible reaction conditions for Suzuki and Sonogashira cross-coupling reactions and for reverse amide bond formation

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ABSTRACT

Progress in DNA-encoded chemical library synthesis and screening crucially relies on the availability of DNA-compatible reactions, which proceed with high yields and excellent purity for a large number of possible building blocks. In the past, experimental conditions have been presented for the execution of Suzuki and Sonogashira cross-coupling reactions on-DNA. In this article, our aim was to optimize Suzuki and Sonogashira reactions, comparing our results to previously published procedures. We have tested the performance of improved conditions using 606 building blocks (including boronic acids, pinacol boranes and terminal alkynes), achieving >70% conversion for 84% of the tested molecules. Moreover, we describe efficient experimental conditions for the on-DNA synthesis of amide bonds, starting from DNA derivatives carrying a carboxylic acid moiety and 300 primary, secondary and aromatic amines, as amide bonds are frequently found in DNA-encoded chemical libraries thanks to their excellent DNA compatibility.

1. Introduction

DNA-encoded chemical libraries (DELs) are collections of organic compounds, individually tagged with DNA fragments, serving as amplifiable identification barcodes.[1–9] DEL technology is gaining increasing popularity, both in industry and academia, thanks to the possibility of synthesizing combinatorial libraries of unprecedented size in an inexpensive fashion and to screen these compound collections against a variety of protein targets.[10–19]

The synthesis of DELs crucially relies on the availability of efficient and DNA-compatible reactions,[20,21] which proceed with good conversion yields for hundreds of structurally-related building blocks, preserving DNA integrity.[22] Among the many reactions that can be considered, Suzuki and Sonogashira cross-coupling, as well as amide bond formation, stand out as particularly attractive procedures. Suzuki and Sonogashira reactions allow the formation of carbon-carbon bonds, which remain a cornerstone in synthetic organic chemistry.[23–27] Amide bonds are found in virtually all DELs reported so far.[10,12–14,28–30] Typically, amides are generated by reaction of an amine (on-DNA) with carboxylic acids or with their reactive derivatives.[21,31,32] However, there is a need for efficient synthetic methods, leading to the formation of amide bonds in a reverse fashion (i.e., by reacting carboxylic acids on-DNA with primary amines).[10]

Our group and other groups have previously described experimental conditions for the execution of Suzuki and Sonogashira couplings on-DNA.[21,24,25,27,33,34] These reactions are important not only because they can proceed with high yield and good DNA integrity if conducted according to suitable protocols, but also because of the large number of aromatic and heteroaromatic halides, boronates and terminal alkynes that can be purchased from commercial sources. We had coupled a set of 32 boronic acids was coupled to a iodophenyl-modified oligonucleotide using 0.8 equivalents of Palladium acetate [$Pd(OAc)_2$] / 3,3′,3″- phosphanetriyltris(benzenesulfonic acid) trisodium salt (TPPTS) as catalyst and evaluated the reaction performance by analysing the crude products by Ultra-Performance Liquid Chromatography – Mass spectrometry (UPLC-MS).[33] In that first report, we identified 23 compounds that coupled to DNA with conversion yields greater than 70%. Similarly, we reported screening results using “copper-free” Sonogashira cross-coupling conditions between 44 alkynes suitable for DEL construction and a iodophenyl-modified oligonucleotide catalysed by allyl palladium chloride dimer ([$Pd(allyl)Cl]_2$) obtaining conversion yields greater than 70% for 46% of the tested compounds.[33] We have recently applied these

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experimental methodologies for the synthesis of a DEL (called "NF-DEL"), which has yielded potent binders against a panel of different target proteins of biological or pharmaceutical interest.[12,35]

In this article, we have compared the efficiency of dihydrogen dichlorobis(di-*t*-butylphosphinito-kP)palladate(2-) [$Pd(tBu_2POH)_2Cl_2$] vs. $Pd(OAc)_2$ for Suzuki cross-coupling, evaluating different reducing agents [TPPTS and Sodium 2′-dicyclohexylphosphino-2,6-dimethoxy-1,1′-biphenyl-3-sulfonate (ssPhos)]. We have also optimized reaction parameters such as the catalyst loading (from 0.8. to 0.4 equivalents) and the reaction temperature (from 60 °C to 70 °C).[25,33] Furthermore, we have compared the efficiency of "copper-free" and "copper-*co*-catalysed" on-DNA Sonogashira cross-coupling reaction, optimizing catalyst loading and reaction temperature, and we have further extended the screening to a broader spectrum alkynes.[33]

Optimization of reaction conditions for the on-DNA synthesis of amides based on amine derivatives on-DNA and on carboxylic acids has previously been described.[31] These reactions are particularly attractive for DEL synthesis, since thousands of carboxylic acids or their functional reactive derivatives are available from commercial sources. However, fewer reports describe procedures for "reverse" amide bond forming reactions, starting from carboxylic acids on-DNA and amines.[19]

In 2020, our group had described the synthesis and the characterization of a DNA-encoded chemical library based on an azido glutamic acid derivative functionalized with two sets of building blocks.[10] The first series of building blocks added to the nascent library corresponded to a set of amines (primary, secondary, aromatic and heteroaromatic), which were coupled to the library scaffold carboxylic acid functional group via reverse amide bond. The final library was screened and yielded ligands multiple protein targets such as PI3K. AASS, HSA, CREBBP, CAIX and FAN-1.[10,12]

In this article, in addition to the optimization of Suzuki and Sonogashira couplings, we have screened three hundred amines for their ability react with a carboxylic acid moiety on-DNA. The carboxylic acid was activated with DMT-MM, a milder activating agent compared to our previously reported EDC / S-NHS activation method.[31] The optimized conditions, featuring the use of and conducted in MOPS buffer at pH = 8.0 instead of TEA buffer at pH = 10.0, yielded conversion rates greater than 70% for 37% of the amines screened.

2. Methods

2.1. Materials and instruments

Amino-modified oligonucleotides were purchased from LGC Ltd. Boronic acids and pinacol boranes were purchased from Apollo Scientific. Alkynes, primary and secondary amines were purchased from Enamine. Palladium (II) salts, copper (II) salts and phosphines (TPPTS and ssPhos) were purchased from ABCR or Sigma-Aldrich. The crude reactions were analysed by Ultra-performance liquid chromatography (UPLC) on 2.1 × 50 mm Acquity BEH300 C4 1.7 μm column (Waters) coupled to an ESI-Tof-MS Waters Xevo G2-XS QTof instrument. The data were analyzed by Waters Masslynx software and the database of building blocks was managed by InstantJChem (ChemAxon).

2.2. Synthesis of Ar-I oligonucleotide conjugate

The amino-modified 14-mer oligonucleotide (MS = 4′473 Da) was coupled to a 3-(4-iodophenyl)propanoic acid as previously reported.[12,13,31]

2.3. Synthesis of "free carboxylic acid" oligonucleotide conjugate

300 different 5′ amino-modified 43-mers were purchased from LGC Ltd and coupled to a (S)-3-azido-4-methoxy-4-oxobutanoic acid as previously reported.[10] The Methyl ester protecting group was removed by treating the oligonucleotide conjugates with 200 mM LiOH water solution.[10]

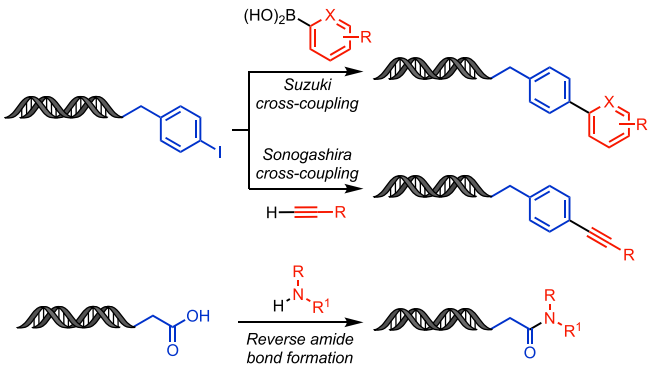


Fig. 1. Schematic representation of on-DNA Suzuki and Sonogashira cross-coupling reactions and on-DNA reverse amide bond formation.

2.4. On-DNA Suzuki cross-coupling

The catalyst solution was prepared by mixing 10 mM $Pd(OAc)_2$ in DMA (40 μL), 100 mM TPPTS (100 μL) in water and water (460 μL). A 1 mM solution of the aryl-iodide functionalized oligonucleotide (10 μL), 500 mM K_2CO_3 buffer (10 μL), the catalyst solution (6 μL, 0.67 mM Pd^0) and a 100 mM boronic acid solution in DMA (20 μL) were combined and heated at 70 °C for 2 h. The reaction was quenched after the addition of 200 mM DTT (5 μL) and 3 M NaOAc/AcOH pH = 4.7 buffer (5 μL). The product was precipitated by the addition of cold ethanol (168 μL).

2.5. On-DNA Sonogashira cross-coupling

The pre-catalyst solution was prepared by mixing 10 mM $Pd(OAc)_2$ in DMA (100 μL), 100 mM TPPTS (100 μL) in water, 20 mM $CuSO_4$ in water (100 μL) and water (700 μL). A 1 mM solution of the aryl-iodide functionalized oligonucleotide (10 μL), 500 mM K_2CO_3 buffer (10 μL), the pre-catalyst solution (6 μL, 1 mM Pd^0 and 2 mM Cu(II)) and a 100 mM alkyne solution in DMSO (20 μL) were combined. The reaction was activated by the addition of 50 mM ascorbate solution (5 μL) and heated at 75 °C for 2 h. The reaction was quenched after the addition of 200 mM DTT (5 μL) and 3 M NaOAc/AcOH pH = 4.7 buffer (5 μL). The product was precipitated by the addition of cold ethanol (183 μL).

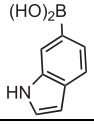
2.6. On-DNA reverse amide bond formation (primary and secondary amines)

A 1.2 mM free carboxylic acid-bearing oligonucleotide solution (40 μL), 100 mM MOPS / 1 M NaCl pH = 7 buffer (137 μL) and 500 mM DMT-MM in 100 mM MOPS / 1 M NaCl pH = 7 buffer (30 μL) were combined and the resulting solution was stirred for 30 min at 30 °C. Afterwards, a 100 mM amine solution in DMSO (75 μL) was added and the reaction was kept at 30 °C for additional 16 h. The product was precipitated by the addition of 5 M NaCl (28 μL) and cold ethanol (775 μL).

2.7. On-DNA reverse amide bond formation (aromatic and heteroaromatic amines)

A 1.2 mM free carboxylic acid-bearing oligonucleotide solution (40 μL), 100 mM MOPS / 1 M NaCl pH = 7.0 buffer (137 μL) and 500 mM DMT-MM in 100 mM MOPS / 1 M NaCl pH = 7 buffer (30 μL) were combined and the resulting solution was stirred for 30 min at 30 °C. Afterwards, a 100 mM amine solution in DMSO (75 μL) was added and the reaction was kept at 45 °C for additional 16 h. The product was precipitated by the addition of 5 M NaCl (28 μL) and cold ethanol (775 μL).

Table 1
Reaction conversions of three representative boronates using different Pd-catalysts (1.0 eq. with respect to DNA-Ar-I): $Pd(OAc)_2$:TPPTS (Cat. 1), $Pd(OAc)_2$:ssPhos (Cat. 2), $Pd(tBu_2POH)_2Cl_2$:TPPTS (Cat. 3) and $Pd(tBu_2POH)_2Cl_2$:ssPhos (Cat. 4).

	Substrate	entry 1 Cat. 1	entry 2 Cat. 2	entry 3 Cat. 3	entry 4 Cat. 4
1		0% [a] 0% [b]	0% [a] 0% [b]	22% [a] 20% [b]	9% [a] 0% [b]
2		30% [a] 0% [b]	23% [a] 0% [b]	100% [a] 100% [b]	30% [a] 14% [b]
3		100% [a] 100% [b]	23% [a] 0% [b]	100% [a] 100% [b]	30% [a] 33% [b]

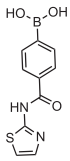
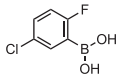
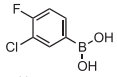
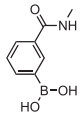
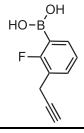
[a] The reaction was carried out at 50 °C.
[b] The reaction was carried out at 60 °C.

3. Results

This report describes the optimization of on-DNA Suzuki cross-coupling, on-DNA Sonogashira cross-coupling as well as the reactivity of amines towards a free carboxylic acid coupled to DNA. We have chosen to focus on these transformations as they are very versatile to DEL synthesis (Fig. 1). Specifically, we have used *p*-iodophenyl derivatives for reaction with (hetero)aromatic boronates (as well as alkene boronate derivatives) or with terminal alkynes. We have also optimized reaction conditions for the coupling of carboxylic acids on-DNA and primary, secondary, aromatic and hetero-aromatic amines.

We started by implementing previously published reaction conditions for the execution of Suzuki cross-coupling, using in total 433 different building blocks, including boronic acids and pinacol boranes.[25,33] In order to optimize water-compatible reaction conditions, we tested different combination of palladium (II) salts [$Pd(OAc)_2$ and $Pd(tBu_2POH)_2Cl_2$], phosphines (TPPTS and *ss*Phos) and temperature (50 °C and 60 °C) using three representative boronic acids with low (Table 1,**1**), medium (Table 1,**2**) and high (Table 1,**3**) yields respectively. We observed that the combination $Pd(tBu_2POH)_2Cl_2$ / TPPTS yielded the best results (Table 1, **entry 3**).

Table 2
Impact of the temperature and catalyst on the conversion yields of seven representative boronic acids. Cat. 1: $Pd(OAc)_2$:TPPTS; Cat. 3: $Pd(tBu_2POH)_2Cl_2$:TPPTS.

	Substrate	Cat. 1, 60 °C	Cat. 3, 60 °C	Cat. 1, 70 °C	Cat. 3, 70 °C
1		60%	0%	98%	12%
2		33%	0%	98%	5%
3		40%	0%	98%	95%
4		35%	0%	98%	82%
5		40%	0%	98%	0%
6		50%	0%	90%	70%
7		43%	0%	98%	15%

The palladium loading was 1.0 eq. in each reaction with respect to DNA.

When we extended the screening to 189 additional compounds, the methodology did not proceed with acceptable yields, since only 38% of substrates had a conversion yield >70% (Fig. 2**a**). Similarly, experimental conditions (that our group had previously published for Sonogashira reactions)[33] were tested using 44 alkynes and we noticed that 70% conversion could be achieved for less than 50% of the tested compounds (Fig. 2**b**). These findings motivated us to further explore improved on-DNA cross-coupling methodologies.

We investigated the impact of temperature on Suzuki reaction

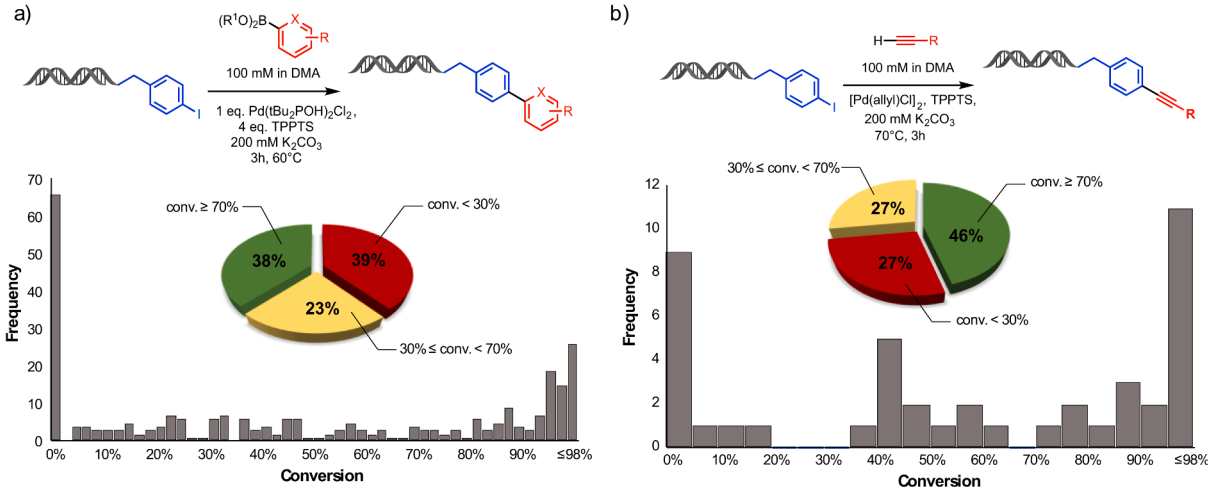
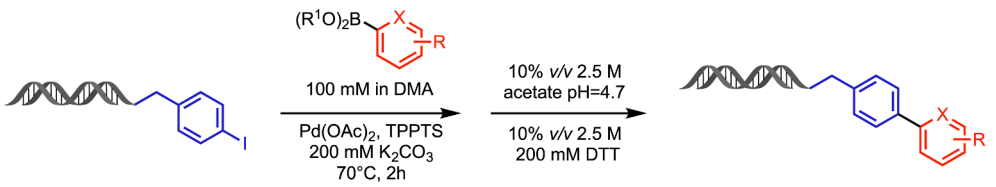
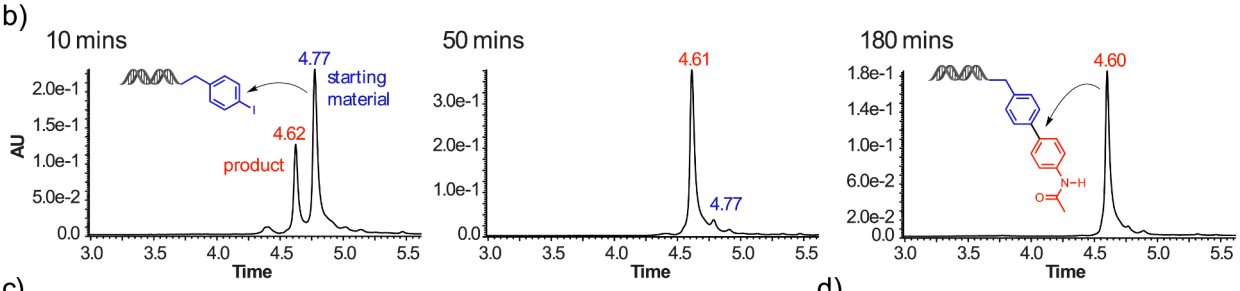


Fig. 2. Histograms represent the distribution of conversions for a) on-DNA Suzuki cross-couplings catalyzed by Pd(tBu2POH)2Cl2 and b) on-DNA Sonogashira cross-couplings catalyzed by [Pd(allyl)Cl]2 before optimizing reaction conditions. The coupling efficiency are also summarized using pie-charts whit in red the fraction of compounds with low conversions (lower than 30%), in yellow the fraction of compounds with intermediate conversions (between 30% and 70%) and in green the fraction of compounds with high conversions (greater then 70%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

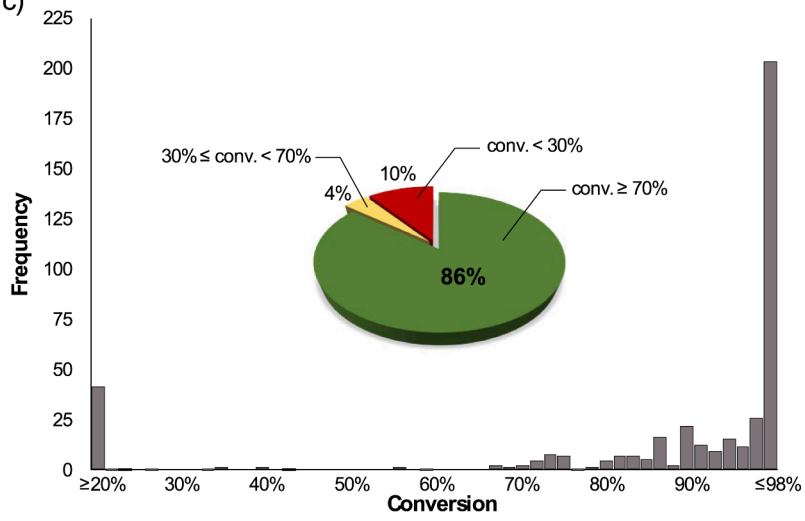
a)



b)



c)



d)

conversion	Pd(tBu2POH)2Cl2		Pd(OAc)2	
20% < conv. ≤ 30%	19	7%	3	1%
30% < conv. ≤ 40%	21	7%	5	1%
40% < conv. ≤ 50%	17	6%	2	0%
50% < conv. ≤ 60%	15	5%	2	0%
60% < conv. ≤ 70%	10	3%	13	3%
70% < conv. ≤ 80%	14	5%	30	7%
80% < conv. ≤ 90%	26	9%	68	16%
90% < conv. ≤ 98%	67	23%	268	62%
Screened cpds	189		391	

e)

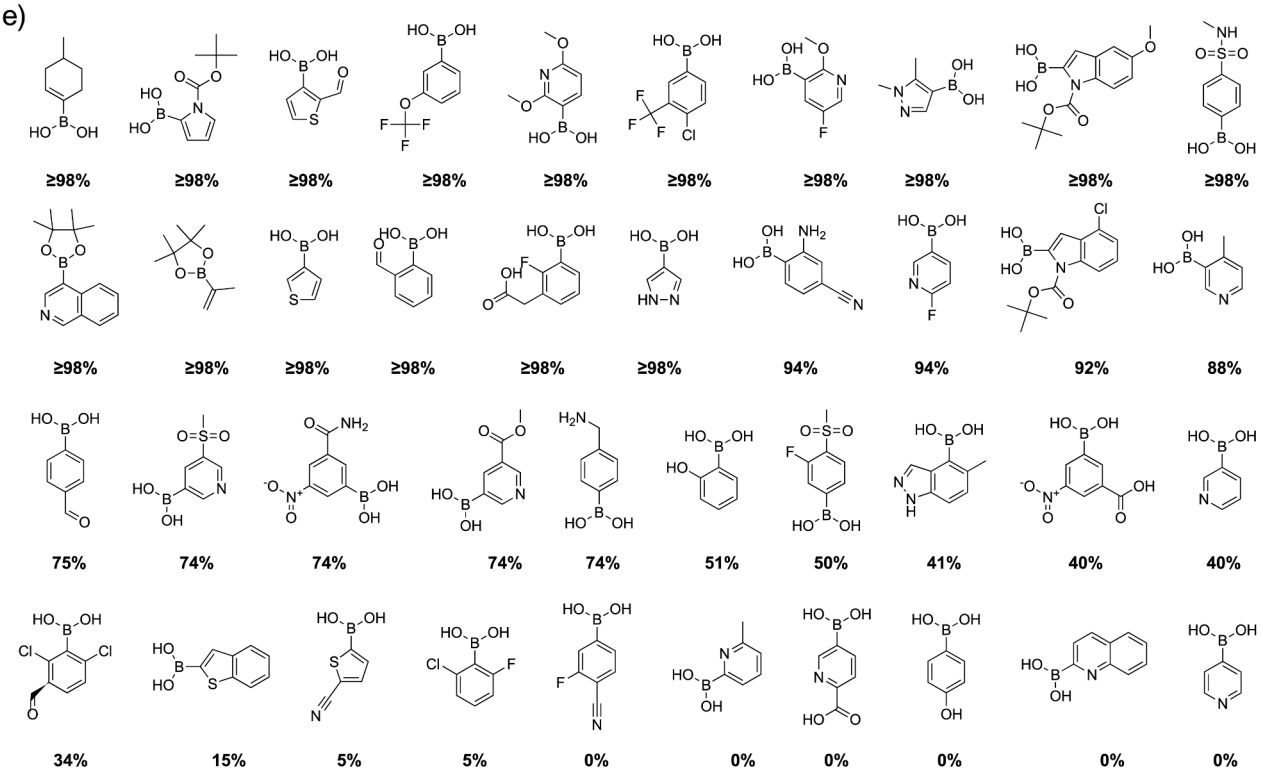


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Fig. 3. On-DNA Suzuki cross-coupling reaction after optimization. a) reaction scheme of Suzuki between 3-(4-iodophenyl)propanoic acid-modified 14-mer oligonucleotide and aromatic or heteroaromatic boronic acid or pinacol boranes. b) LC traces of reaction after 10, 50 and 180 min of the starting material highlighted in blue and the cross-coupling product highlighted in red. c) the histogram and the pie-chart represent the distribution of conversions of Suzuki cross-coupling with the optimized conditions. d) These results are also reported in a table and compared with the results obtained by using Pd(tBu2POH)2Cl2 / TPPTS catalyst. Both reactions were carried out at 70 °C. e) Some representative aliphatic, aromatic and heteroaromatic boronates with the corresponding conversion yield indicated. All screened boronic acids and pinacol boranes are reported in **Supplementary** Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conversion by increasing it by 10 °C (from 60 °C to 70 °C) using both palladium acetate and $Pd(tBu_2POH)_2Cl_2$ in combination with TPPS (Table 2). We observed a substantial improvement of reaction conversions and a wider range of building block compatibility when Palladium acetate was used as catalyst and TPPTS as palladium (II) reducing agent. Finally, the reactions were scaled up to 10 nmol of starting material (*p*-iodophenyl DNA derivative) and the catalyst loading was lowered down to 0.4 equivalents.

Fig. 3**a** reports the experimental conditions for the optimized Suzuki coupling procedure, which was found to perform well on a wide range of different substrates. The *p*-iodophenyl propionic acid oligonucleotide conjugate (14-mer) in carbonate buffer was incubated with a series of boronic acids (200 equivalents) and with palladium acetate / TPPTS catalyst (0.4 equivalents of Pd). The reactions were heated at 70 °C for 2 h and quenched by the addition of DTT (100 equivalents) and acetate buffer. Fig. 3**b** shows the experimental methodology that was used to monitor the progression of the reactions and determine conversion yields. In this example, an aryl iodide on-DNA was reacted with (4-acetamidophenyl)boronic acid. The reaction was tested by UPLC at various time points and we could detect the progressive conversion of the starting material (still visible after 10 min) into the desired product (which was quantitatively obtained after 180 min). Mass spectrometric analysis confirmed the identity and integrity of the products. Fig. 3**c** presents a pie chart, indicating the proportion of substrates that had reacted with a *p*-iodophenyl derivative on-DNA with a yield of <30% [10% of substrates], between 30 and 70% [4% of substrates] or more than 70% [86% of substrates]. The improved reaction conditions were tested on a total of 433 molecules, thus providing a robust experimental basis for subsequent library construction activities.[12] In addition to the pie chart, a histogram provides a more detailed readout regarding the distribution of conversion yields for the tested molecules. Fig. 3**d** reports a comparison between a $Pd(tBu_2POH)_2Cl_2$ / TPPTS catalyzed and palladium acetate / TPPTS catalyzed reaction, both carried out at 70 °C using 1 and 0.4 equivalents of palladium, respectively. The reaction catalyzed by palladium acetate and TPPTS showed a superior performance in terms of conversion for a majority of boronic acids or pinacol boranes (85% vs. 37% of compounds with conversion >70%). A few examples of boronate derivatives used for our screening campaign are reported in Fig. 3**e**. The complete list of compounds and their corresponding coupling conversion is reported in **Supplementary** Table 1.

Similar to what was previously described, optimization experiments (e.g. catalyst, catalyst loading and reaction temperature) were performed using an on-DNA Sonogashira cross-coupling procedure. The main improvement was observed when allyl palladium(II) chloride dimer was substituted with a palladium (0) – copper (I) based catalyst.

Fig. 4**a** shows the experimental conditions for the Sonogashira coupling procedure, optimized on a large set of terminal alkynes in the presence of a palladium (0) – copper (I) catalyst. The aryl-iodide DNA derivative was incubated with 100 mM alkyne in the presence of 0.6 equivalents of palladium acetate, TPPTS and 1.2 equivalents of copper (II) sulfate. The catalyst was activated by reducing Cu(II) to Cu(I) with an excess of sodium ascorbate. The reactions were followed over time by UPLC-MS as described for Suzuki cross-coupling. Fig. 4**b** presents a pie chart, indicating the proportion of terminal alkynes that had reacted with a *p*-iodophenyl derivative on-DNA with a yield of < 30% [6% of substrates], between 30 and 70% [9% of substrates] or more than 70% [85% of substrates]. The optimized protocol was tested on 173 molecules, >150 of which resulted in excellent yields for DEL library construction.[12] In addition to the pie chart, a histogram provides a more detailed readout regarding the distribution of conversion yields for the tested molecules. The table reported in Fig. 3**c** shows a detailed comparison between "copper-free"[33] and the optimized "copper co-catalyzed" Sonogashira cross-coupling in terms of conversions. Some examples of tested alkynes and their corresponding conversions are reported in Fig. 4**d**. The complete list of compounds and their corresponding coupling conversion is reported in **Supplementary** Table 2.

As previously mentioned, we also aimed at improving reaction conditions for the transformation of carboxylic acids on-DNA with amines to form amides. Fig. 5**a** illustrates the experimental conditions for the reverse amide coupling between a set 41-mer oligonucleotides modified with a (*R*,*S*)-2-azidopentanedioic acid moiety and a set of primary and secondary amines.

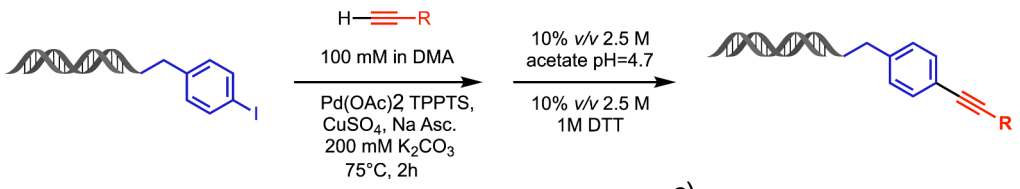
The amines were incubated with pre-activated oligonucleotides for 16 h at 30 °C. Similarly, Fig. 5**b** shows the reaction conditions developed for the coupling of aromatic and heteroaromatic amines, which resulted more difficult to couple compared with primary and secondary amines. In order to increase the conversions, the reactions were incubated at 45 °C for 16 h. Fig. 5**c** shows a pie chart reporting the proportion of substrates that reacted with (*R*,*S*)-2-azidopentanedioic acid oligonucleotide derivatives with a yield of < 30% (11% of substrates), between 30 and 70% (52% of substrates) or more than 70% (37% of substrates). These reaction conditions were tested on 300 building blocks, including primary, secondary and aromatic/heteroaromatic amines and subsequently used for DEL library construction (Table 3).[10] The histogram shows the distribution of conversions of amines. Some representative structures of amines which were screened are reported in Fig. 5**d**. The complete list of building blocks and the corresponding coupling conversions is reported in **Supplementary** Table 3.

4. Discussion

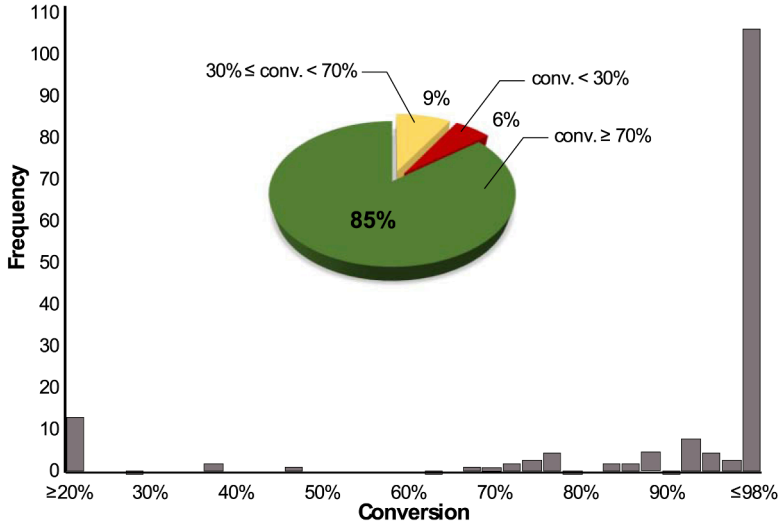
In this work, we have aimed at presenting a detailed account on our activities focused on the optimization and characterization of robust chemical transformation, for DEL synthesis. We chose to work on Suzuki cross-coupling, Sonogashira reaction and on reverse amide formation, as these transformations can be performed in a robust manner, with an extremely broad scope of starting reagents. All three reactions have previously been used by our group for the construction of well-performing DELs.[10]

The on-DNA optimization of Suzuki cross-coupling reactions has been the topic of numerous investigations.[21,24,25,33,34] We felt that there was still room for improvement, since some structures did not react well under conventional conditions. The use of palladium acetate and TPPTS at 70 °C in potassium carbonate buffer led to conversion yields greater than 70% for the majority of building blocks, when the conditions were tested in over 433 compounds. In our experience, it is good practice to use a sufficiently large number of test reactions, in order to gain confidence about the performance of the technology for DEL synthetic purposes. Most building blocks yielded full conversions under optimized reaction conditions. However, a small proportion of boronates, such as those featuring pyridine derivatives (with *ortho*- and *para*-boronic acid modification), electron-poor structures (e.g., those with di- or trisubstituted aromatic rings with halides or nitro derivatives), insoluble or sterically-hindered molecules failed to give acceptable conversion yields, even in optimized conditions. Nonetheless, 86% of the boronates that we had tested gave at least 70% conversion in Suzuki coupling.

a)



b)



c)

conversion range	copper free		copper co-cat.	
≤ 20%	12	27%	14	8%
20% < conv. ≤ 30%	0	0%	1	1%
30% < conv. ≤ 40%	1	2%	3	2%
40% < conv. ≤ 50%	7	16%	2	1%
50% < conv. ≤ 60%	3	7%	1	1%
60% < conv. ≤ 70%	1	2%	7	4%
70% < conv. ≤ 80%	3	7%	13	8%
80% < conv. ≤ 90%	4	9%	14	8%
90% < conv. ≤ 98%	13	30%	118	68%
Screened cpds	**44**		**173**	

d)

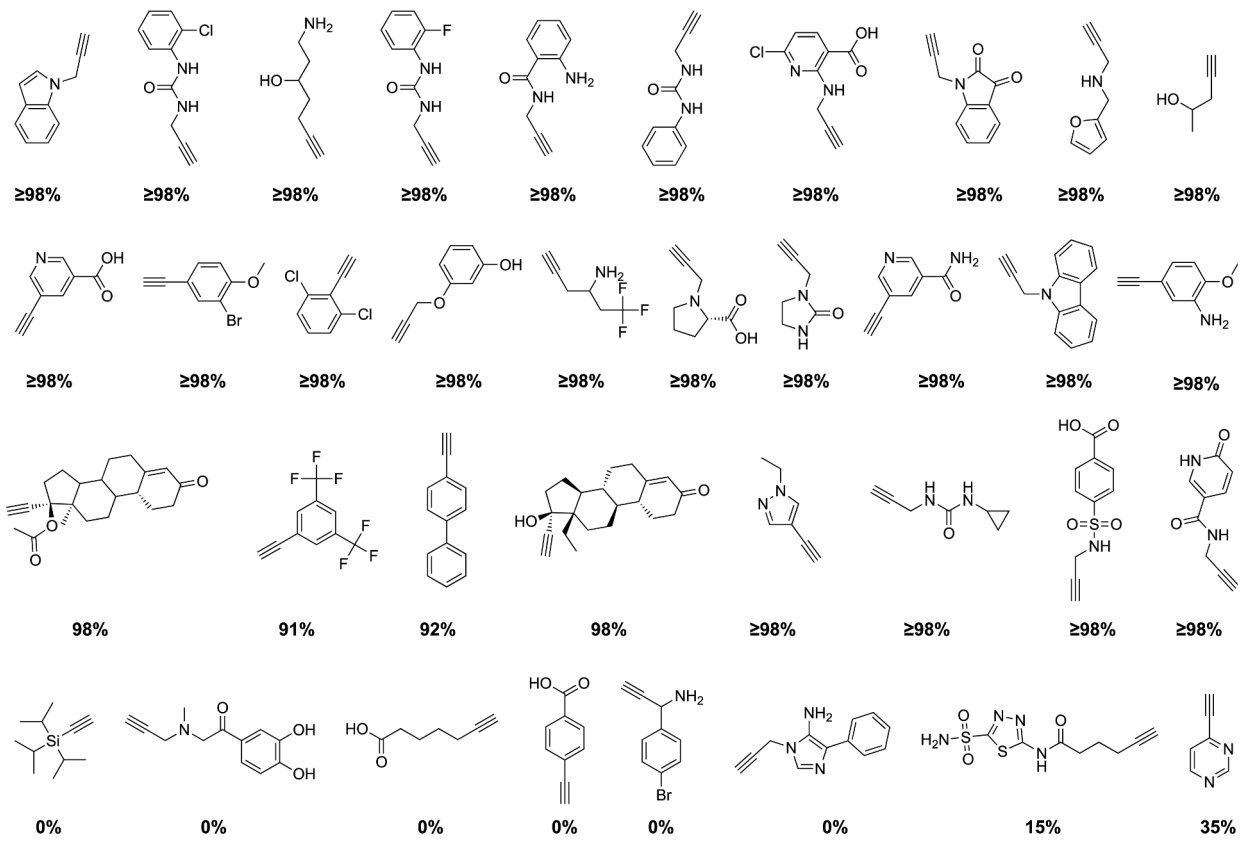


Fig. 4. On-DNA "copper co-catalyzed" Sonogashira cross-coupling reaction after optimization. a) reaction scheme of Sonogashira between 3-(4-iodophenyl)propanoic acid-modified 14-mer oligonucleotide and terminal alkynes. b) the histogram and the pie-chart represent the distribution of conversions of Sonogashira cross-coupling with the optimized conditions. c) These results are also reported in a table and compared with the results obtained before conditions were optimized (copper-free Sonogashira). d) Some representative terminal alkynes with the corresponding coupling conversions indicated. All screened alkynes are reported in **Supplementary** Table 2.

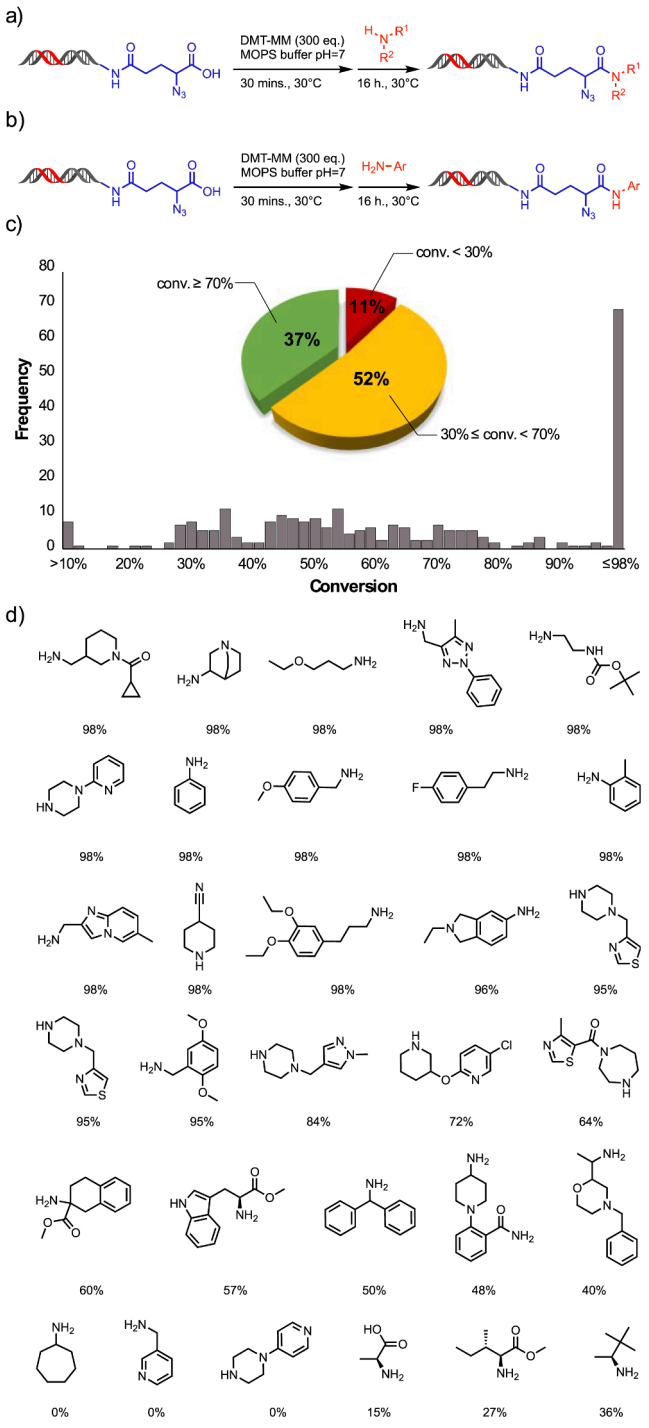


Fig. 5. On-DNA reverse amide bond formation reaction. a) reaction scheme of On-DNA reverse amide bond formation between (*R*,*S*)-2-azidopentanedioic acid modified 43-mer oligonucleotides and primary or secondary amines. The coding region of the set of oligonucleotides is highlighted in red. b) reaction scheme of On-DNA reverse amide bond formation between (*R*,*S*)-2-azidopentanedioic acid modified 43-mer oligonucleotides and aromatic amines. The coding region of the set of oligonucleotides is highlighted in red. c) the histogram and the pie-chart represent the distribution of conversions of on-DNA amide bond formation reaction for primary, secondary and aromatic amines. d) Some representative primary, secondary and aromatic amines with the corresponding coupling conversions indicated. All the screened amines are reported in the **Supplementary** Table 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Conversion yields for different classes of amines.

	primary amines		secondary amines		aromatic/ heteroaromatic amines	
conv. ≥ 70%	66	41%	28	29%	17	40%
30% ≤ conv. < 70%	77	48%	56	58%	22	51%
conv. < 30%	18	11%	12	13%	4	9%
total	**161**		**96**		**43**	

In general, the performance of Sonogashira cross-coupling achieved at 75 °C in carbonate buffer, using palladium acetate / TPPTS and copper sulfate / sodium ascorbate as catalyst, is very good. Approximately 85% of terminal alkynes gave conversion yields greater than 70%. Few structures reacted less well, including catechols and resorcinols (which may chelate metals), as well as insoluble or sterically-hindered compounds (e.g. ethynyltriisopropylsilane, 1-ethynylpyrene, etc.).

The most challenging reaction to implement with a high tolerance for chemical variations was the reverse amide bond formation, in which a carboxylic acid coupled to a DNA oligonucleotide would be reacted with amines to form the corresponding amide moieties. In our hands, after having tested 300 different amines, we found that 37% of them would give conversion yields greater than 70%, while the remaining 63% would exhibit any yield between 10 and 70%. In our hands, it was difficult to activate a carboxylic acid on-DNA. Strong activators should be avoided, as they could modify functional groups at the DNA bases. As expected, the worst reactivities were observed for free amino acids, electron-poor amines and those containing deactivating substituents (e.g., aromatic or heteroaromatic amines).

5. Conclusion

We have described experimental procedures that lead to the execution of on-DNA Suzuki cross-coupling, Sonogashira cross-coupling and reverse amide bond formation with high conversion yields. The methodologies have been tested using more than 900 substrates and have shown to proceed with >70% yield for 665 building blocks. Moreover, we have implemented those reactions for the "real-life" synthesis of DEL, from which valuable binding molecules have been isolated. In spite of these achievements, 27% of tested building blocks have shown insufficient reactivity in our studies. This finding suggests that there may still be a need for alternative reaction conditions, that may be used for a subset of "difficult-to-react" building blocks.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: D. N. is co-founder and shareholder of Philochem (www.philochem.com), a company active in the field of DNA-encoded chemical libraries.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116206.

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