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Expedient construction of small molecule macroarrays *via* sequential palladium- and copper-mediated reactions and their *ex situ* biological testing†

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Abstract

We report the highly efficient syntheses of a series of focused libraries in the small molecule macroarray format using Suzuki–Miyaura and copper-catalyzed azide–alkyne cycloaddition (or “click”) reactions. The libraries were based on stilbene and triazole scaffolds, which are known to have a broad range of biological activities, including quorum-sensing (QS) modulation in bacteria. The library products were generated in parallel on the macroarray in extremely short reaction times (~10–20 min) and isolated in excellent purities. Biological testing of one macroarray library post-cleavage (*ex situ*) revealed several potent agonists of the QS receptor, LuxR, in *Vibrio fischeri*. These synthetic agonists, in contrast to others that we have reported, were only active in the presence of the native QS signal in *V. fischeri*, which is suggestive of a different mode of activity. Notably, the results presented herein showcase the ready compatibility of the macroarray platform with chemical reactions that are commonly utilized in small molecule probe and drug discovery today. As such, this work serves to expand the utility of the small molecule macroarray as a rapid and operationally straightforward approach toward the synthesis and screening of bioactive agents.

Introduction

Small molecules are valuable tools to explore biological phenomena and methods that permit the rapid synthesis and biological testing of such compounds are highly sought-after in chemical biology.^{1–3} The small molecule macroarray represents one such approach for the synthesis and identification of novel bioactive agents.^{4,5} Macroarrays are readily constructed *via* the SPOT-synthesis technique on a planar cellulose support,⁶ yielding spatially addressed libraries of ~10–1000 unique compounds (Scheme 1). These cellulose membranes are straightforward to manipulate, remarkably mechanically robust, and compatible with a wide range of *in situ* and *ex situ* biological assay formats performed either on or off the planar support, respectively.⁴ The technical simplicity and overall economy of macroarray construction renders this method of parallel library synthesis especially attractive in academic and basic research settings.⁷

Despite these advantages, up until a few years ago, the small molecule macroarray had been applied chiefly in the synthesis of peptidic derivatives based on simple acylation-type

†Electronic supplementary information (ESI) available: Full macroarray synthesis details, compound characterization data, and biological protocols.

chemistry.^{8–10} As the small molecule macroarray evolved from the SPOT-synthesis technique, this paucity of application is perhaps not surprising, as SPOT-synthesis was developed primarily as a tool for the construction of peptide arrays.¹¹ Our laboratory recognized the potential broad utility of the small molecule macroarray if it could be applied to targets beyond peptides, and over the last several years we have systematically studied the scope and limitations of a range of chemical reactions on planar cellulose supports. Prior to this work, the limited study of more challenging chemical reactions on planar cellulose supports can be attributed, in part, to (1) the slow reaction rates on heterogeneous supports and the inability to use stirring, (2) the necessity for extremely short reaction times (< 15 min) due to rapid solvent evaporation post-spotting and (3) concerns about the stability of cellulose supports under certain reaction conditions.⁴

Our initial work focused on circumventing the slow reaction rates yet obtaining requisite short reaction times on planar cellulose supports, and we found that microwave-assisted organic reactions were ideally suited to macroarray synthesis.^{12–16} These reactions were complete in ~10–15 min at 80–100 °C, promoted by the polar, high boiling solvents that display slowed evaporation, and were easily performed in commercial multi- and monomodal microwave reactors.⁴ Short reaction times were also achievable using conventional heating sources (*i.e.*, in a drying oven) under certain conditions.¹³ We later found that numerous organic reactions were compatible with the cellulose supports, including Aldol condensations,^{12,17} various nitrogen- and oxygen-containing heterocycle-forming reactions,^{13,15,18,19} and Ugi four-component reactions.^{14,16} Several optimized support systems have been developed, with the acid-cleavable Rink-amide linker systems being some of the most robust (Scheme 1).^{17,20} Through these studies, we have found that the planar support can withstand at least 10–15 sequential reactions and washing steps and is resilient to a range of solvents, temperatures (up to 125 °C) and pH values (1–14). These features, coupled with extremely short reaction times (minutes) render the macroarray platform compatible with a wide variety of methods for the focused parallel synthesis of small molecules beyond peptides.

Our current goal for the macroarray synthesis platform is to demonstrate its compatibility with an expanded set of chemical reactions that are most typical in small molecule probe and drug discovery today. Metal-catalysed organic transformations feature prominently within this subset of chemical reactions,²¹ with palladium-catalyzed C–C and C–N bond forming reactions²² and the Huisgen copper-catalyzed azide–alkyne cycloaddition (CuAAC or “click”) reaction^{23,24} receiving significant recent attention. Toward this goal, we recently reported a study of the use of palladium-catalyzed Heck reactions in small molecule macroarray synthesis. We found that the reaction was highly compatible with the cellulose support and we generated libraries of stilbene derivatives in high purities in short overall reaction times.²⁰ This study represented the first application, to our knowledge, of a metal-mediated reaction on the small molecule array platform. In the current study, we sought to build on this initial work and further enlarge the repertoire of metal-mediated reactions applied in the macroarray format.

Here, we report highly efficient protocols for small molecule macroarray synthesis *via* the palladium-catalyzed Suzuki–Miyaura reaction²⁵ and the CuAAC “click” reaction.^{26,27} Both reactions signify reliable methods to connect readily accessible building blocks in the presence of a wide range of functional groups.²⁸ Several libraries of novel phenyl-naphthalenes and triazoles were constructed and we also demonstrate the first example of sequential metal-mediated reactions on the macroarray platform through the synthesis of a small library of Suzuki–Miyaura/CuAAC products. Preliminary off-support biological testing revealed that certain library members were extremely strong agonists in bacterial cell–cell signalling assays. Together, these results serve to not only underscore, but also

broaden the utility of the small molecule macroarray for the targeted synthesis and discovery of non-peptidic, bioactive agents.

Results and discussion

Macroarray synthesis via the Suzuki–Miyaura reaction

We commenced our studies by first investigating the compatibility of the Suzuki–Miyaura reaction on the macroarray platform. We selected a phenyl-naphthalene scaffold (**1**) for focused library design (Fig. 1A), as such stilbene-type (**2**) derivatives are accessible *via* the Suzuki–Miyaura reaction and show a broad range of biological activities.²⁹ Phenyl-naphthalenes (**1**) preserve the stilbene framework, yet lack the chemical and metabolic instability inherent to the stilbene double bond.^{30,31,32} Notably, our group and others have shown that several stilbenes (**2**) inhibit bacterial cell–cell signalling (or quorum sensing (QS)) in Gram-negative bacteria.^{20,33–35} QS is widespread in bacteria,³⁶ and plays a major role in virulence and infection.^{37,38} How stilbenes elicit their QS antagonistic activities remains unknown and therefore further study of this class compounds is of interest.

We utilized the robust Rink-amide linker derivatized cellulose (**3**) as our planar support system for study of the Suzuki–Miyaura reaction (Fig. 1B). We have previously reported the use of support **3** for the successful synthesis of several macroarrays.^{17,20} Support **3** contains a flexible amine spacer between the support and the linker, and the linker is applied in a spatially addressable format (*i.e.*, in 0.3 cm² spots, 12 × 12 mm apart).³⁹ The Rink-amide linker is *N*-Fmoc protected for long-term support storage and is deprotected under basic conditions immediately prior to loading the first library building block. Compound cleavage for loading and product conversion analyses can be easily affected under acidic conditions; we have found that the use of acid vapour (TFA) for spatially addressed “dry” cleavage is the most convenient (Scheme 1).

The Suzuki–Miyaura reaction requires an alkyl halide and a boronic acid coupling partner.²⁵ Thus, either of these building blocks could theoretically be appended to support **3** to initiate macroarray synthesis. Our initial experiments focused on spotting a pre-mixed solution of aryl iodide and Pd(OAc)₂ (*i.e.*, pipetting 3.0 μL per spot) onto planar support **3** loaded with boronic acids derivative **4c**. This strategy had the advantage of oxidative addition potentially occurring in solution prior to support spotting.⁴⁰ While this approach resulted in good conversions, we discovered that 10–15% of the immobilized boronic acid underwent protodeboronation during the Suzuki–Miyaura reaction, resulting in inadequate product purities. These results led us to reverse our synthetic approach and we investigated the feasibility of the Suzuki–Miyaura reaction between immobilized halides and spotting solutions of boronic acids (such as **5a–b** and **6**, respectively; Fig. 1B) instead.

The 6- and 7-bromo-2-naphthoxyacetic acids (**4a–b**) were synthesized readily in solution (see ESI[†]) and loaded onto amino support **3** using a standard carbodiimide (DIC) coupling reaction (Fig. 1B). We then screened a wide range of spotting solutions containing both a palladium catalyst and aryl boronic acids; disappointingly, all of these conditions gave either low yields of the desired phenyl-naphthalene products or boronic acid decomposition prior to spotting. However, further studies revealed that the Suzuki–Miyaura reaction proceeded remarkably well on cellulose support by spotting the palladium catalyst and the aryl boronic acids onto support **5a–b** *sequentially* in two steps.

We carefully optimized this sequential spotting protocol and found that spotting Pd(OAc)₂ in a 9 : 1 mixture of *N*-methyl-2-pyrrolidone (NMP) and H₂O onto **5a–b**, letting the support sit at room temperature (rt) for 10 min, followed by spotting various aryl boronic acids and Cs₂CO₃ in NMP:H₂O 9 : 1 and heating at 60 °C for 12 min, led to highly efficient C–C

bond formation to yield phenyl-naphthalenes (Fig. 1B). The short reaction time (22 min) and high conversions for this heterogeneous reaction are especially notable; Suzuki–Miyaura reactions performed on traditional solid support beads typically require ~24 h reaction times.^{41,42} Similar to our previous observations for the Heck reaction performed on planar cellulose,²⁰ we found that the Suzuki–Miyaura reaction did not require phosphine type ligands. However, a minimum excess of 1.2 equiv. Pd catalyst (with respect to the immobilized bromide **5a–b**) was needed to achieve full consumption of the aryl bromide. We note that the scale of macroarray synthesis (*i.e.*, nanomoles of compound per spot) does not demand a catalytic process, as very small amounts of reagents are necessary for product conversion. Importantly, no Pd contamination was observed in phenyl-naphthalenes analyzed post-cleavage by inductively coupled plasma (ICP) trace analysis (see ESI†).

We applied the optimized Suzuki–Miyaura reaction protocol to the synthesis of a 70-member phenyl-naphthalene macroarray (Fig. 1B). We prepared a 17.5 × 25 cm array loaded with 35 individual spots of aryl bromides **4a–b**. Next, we performed the Suzuki–Miyaura reaction to couple 35 unique, commercially available aryl boronic acids (**6**) onto support **5a–b**. The phenyl-naphthalene products (**7a,b**) were isolated after spatially-addressed cleavage using TFA vapour, in 100 and 115 nmol per spot quantities (for **7a** and **7b**, respectively).⁴³ HPLC-MS analysis of a representative set of 46 library members showed excellent product conversions and purities, with averages of >98% and >97%, respectively (a subset of these are shown in Table 1; see ESI† for full set). Moreover, the Suzuki–Miyaura reaction protocol worked equally well regardless of the functionality type (electron rich or poor groups; *i.e.*, alcohols, ethers, ketones, esters, tertiary amines, halogens, cyanides and nitriles) and the substitution pattern (*ortho*, *meta*, *para*, di-substituted) on the aryl boronic acid. These results indicate that the small molecule macroarray is highly compatible with the Suzuki–Miyaura reaction and support the application of this reaction for the generation of other libraries in macroarray format.

Macroarray synthesis *via* the CuAAC reaction

A second focus of this study was to explore the feasibility of the widely utilized CuAAC reaction⁴⁴ on the small molecule macroarray platform. Similar to our studies of the Suzuki–Miyaura reaction, we selected the Rink-linker-derivatized cellulose support **3** for array construction (Fig. 2A). Likewise, we needed to determine which of the two reaction building blocks, in this case azides or alkynes, to immobilize on the cellulose support. Our first experiments explored the reactivity of immobilized azides in the CuAAC reaction. These studies quickly revealed poor conversion to the desired triazole and various unwanted oxidative coupling by-products. We thus re-focused on immobilizing the alkyne instead and observed significantly higher conversions and product purities.

A quick catalyst screen identified copper(I) iodide dissolved in pyridine as the optimal catalyst system for the CuAAC on the planar cellulose support. We then undertook a detailed reaction optimization study to identify an efficient and practical CuAAC protocol for macroarray synthesis. The final protocol involved the following (shown in Fig. 2A): (1) immobilization of an alkynyl carboxylic acid (such as **8**) onto support **3** *via* a DIC coupling reaction analogous to that used in the Suzuki–Miyaura reaction above, (2) spotting a solution of an azide (**10**, 1.3 eq.) and copper(I) iodide (1.0 eq.) in pyridine (17 eq.) and 2-acetylpyridine (3.0 eq.) onto the alkyne support **9**, and (3) heating the support at 60 °C for 12 min.⁴⁵ These conditions gave exclusive formation of the desired 1,4-disubstituted 1,2,3-triazole (**11**). The short reaction time was analogous to the Suzuki–Miyaura reaction conditions above (12 min) and, again, atypical of most solid-phase reactions.⁴⁶ The addition of 2-acetylpyridine served to suppress the formation of the main impurity, namely 5-iodo-1,2,3-triazoles. Stoichiometric amounts of Cu were required to achieve full initial

building block consumption. The elevated reaction temperature (60 °C) was necessary to avoid oxidative coupling by-products otherwise observed when the reaction was performed at rt.

We next applied this CuAAC protocol to the synthesis of a 44-member macroarray of 1,4-disubstituted 1,2,3-triazoles (Fig. 2A–C). First, four different alkynyl acids (**8**) were coupled to amino support **3** (11 spots of each, on an 27.5 × 10 cm, array). Next, 11 individual azides (**10**) were spotted onto support **9** under our optimized CuAAC conditions to yield 44 unique triazole products. HPLC-MS analysis of the entire library post-cleavage indicated that these reaction conditions worked universally for all four alkyne building blocks, giving excellent conversions and purities (averages of >98% and >97%, respectively; Table 2).⁴³ Azide functional group diversity was well tolerated and reactivity trends were similar to those previously reported for solution-phase CuAAC reactions, with primary azides exhibiting slightly higher reactivities than secondary and tertiary azides.⁴⁷ In addition, we found that the product conversions were identical over a range of initial alkyne loadings (50–300 nmol per spot of support **9**), allowing for the easy tuning of triazole product quantities. Not all reactions on the macroarray platform are compatible with a range of substrate loadings^{13,19} and, therefore, this result is noteworthy. Furthermore, the ability to tune product quantities is beneficial for certain on-support assays (*e.g.*, protein binding) where controlling the density of immobilized compound is important.⁴

Macroarray synthesis *via* sequential CuAAC and Suzuki–Miyaura reactions

The ability to perform multiple metal-mediated reactions in small molecule macroarray synthesis would provide access to libraries of heightened diversity. Such sequential reaction sequences are yet to be evaluated. We therefore investigated the use of tandem CuAAC/Suzuki–Miyaura reactions for the construction of a small molecule macroarray (Fig. 3) and applied the optimized conditions for each reaction described above. The first part of the synthetic route was analogous to that for triazole macroarray **11** (Fig. 2A), yet we selected only one alkyne (**8-A**) and one azide building block (4-bromobenzyl azide **10-C**) to yield an array of immobilized triazole **12** (as 18 individual spots). The selection of the 4-bromobenzyl azide **10-C** allowed us to subsequently submit the triazole array to the Suzuki–Miyaura reaction with a set of 18 different aryl boronic acids (**6**) to deliver an 18-member biphenyl-derivatized triazole library (**13**, Fig. 3). We were pleased to observe excellent final product conversions and purities for this macroarray (>97 and >94%, respectively; Table 3). These results serve to showcase the compatibility of the small molecule macroarray platform with synthetic routes containing sequential diversity generating chemical steps and suggest that the construction of macroarray libraries of considerably expanded structural complexity relative to previous macroarray syntheses will be feasible.

Biological testing of the phenyl-naphthalene library

As introduced above, several stilbene derivatives have been shown to have QS modulatory activity and a final goal of this study was to examine the activity of the new stilbene-type compounds derived from macroarrays in QS assays. Molecules that intercept bacterial QS pathways and have structures divergent from natural occurring QS signals remain rare.³⁷ We therefore evaluated the phenyl-naphthalene library (**7a–b**) for QS antagonistic activities in the bacterial symbiont *V. fischeri*. This well-studied Gram-negative bacterium utilizes the canonical QS system based on *N*-acyl L-homoserine lactone (HL) signals and LuxR-type receptors to control bioluminescence and thus represented an excellent model organism for study.^{37,48} We utilized a QS mutant strain (*V. fischeri* ES114 $\Delta luxI$) that lacks a functional LuxI synthase; thus, the exogenous addition of an appropriate agonist is required to activate LuxR and LuxR activity is easily measured by luminescence. We examined the ability of the

phenyl-naphthalene library **7a–b** to inhibit LuxR using this strain. Competitive inhibition assays were performed at 2 μ M **7a–b** against 2 μ M of *V. fischeri*'s native QS ligand, *N*-(3-oxo-hexanoyl)-L-HL (OHHL).

We found that, in contrast to our previous results with stilbenes,²⁰ these phenyl-naphthalene derivatives had minimal LuxR antagonistic activity, suggesting that this further rigidified scaffold could diminish ligand activity. However, the antagonism screen revealed several extremely potent LuxR agonists instead (Fig. 4), with activities comparable or higher than some of the most potent known non-native LuxR agonists (e.g., *N*-(3-nitro-phenylacetanoyl)-L-HL (control **C14**)).⁴⁹ Structure–activity relationship analyses indicated that significant agonistic activity (> 30% activation) was only achieved with phenyl-naphthalenes of type **7b** bearing a substituent in the *para*-position on the phenyl group. Most notable amongst this group of activators were the *para*-acetyl (**7b-9**) and *para*-CN (**7b-12**) derivatives (Fig. 4), which were capable of activating LuxR to 411% and 248% relative to OHHL, respectively. Interestingly and in contrast to **C14**, the phenyl-naphthalene LuxR agonists were only active in the presence of OHHL (see ESI† for agonism assay data), suggesting an alternate mechanism of LuxR agonism relative to **C14**. Additional experiments are required to further characterize their mode of action (e.g., the aromatic phenyl-naphthalene behaving as a co-factor mimic is one avenue for study). Nevertheless, these data provide support for the continued study of stilbene analogs as non-native QS modulators. Furthermore, they highlight the utility of the small molecule macroarray for the rapid discovery of new bioactive probe molecules, as the synthesis and screening of library **7a–b** required a total of 16 h of experimental time (from initial cellulose functionalization to bacteriological assay data analysis, with 2 h being unattended assay time).

Conclusions

In summary, we report here the first application of two powerful chemical reactions, the Suzuki–Miyaura and CuAAC reactions, on the small molecule macroarray platform. We demonstrate that these reactions can be implemented, either individually or sequentially, for the efficient construction of focused libraries of phenyl-naphthalenes (**7a–b**) and 1,2,3-triazoles (**11** and **13**) in high purities on rapid time scales. The phenyl-naphthalene scaffold was of particular interest due to the ability of several structurally related stilbenes to modulate bacterial QS pathways. We found that the Suzuki–Miyaura reaction exhibited a broad aryl boronic acid substrate scope on the planar cellulose support. Likewise, the CuAAC reaction was tolerant of a range of structurally divergent alkynes and azides. Perhaps most remarkable were the short reaction times necessary for each of these reactions (~12 min), which is comparable to, if not shorter than typical reaction times under solution-phase conditions.^{41,42,46} Subsequent testing of the phenyl-naphthalene library (**7a–b**) in a bacterial QS reporter assay revealed several novel and potent activators of QS in the Gram-negative bacterium, *V. fischeri*, in the presence of its native QS signal (OHHL). Overall, the results reported herein showcase the compatibility of the macroarray platform with chemical reactions that are commonly utilized in small molecule probe and drug discovery today. Furthermore, our data support the expanding utility of the small molecule macroarray as a rapid and operationally straightforward approach toward the synthesis and screening of bioactive agents. Ongoing work is focused on the continued application of these and other metal-mediated reactions for the synthesis of novel and sizable macroarrays for chemical biology research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

A general scheme of the small molecule macroarray synthesis and screening process. The green circle bound to array compounds in the on-support assays (bottom right) represents a biological target of interest.

Fig. 1.

A) Generic phenyl-naphthalene (**1**) and stilbene (**2**) scaffolds. An arrow indicates the site of the envisaged Suzuki–Miyaura reaction to generate **1**. B) Suzuki–Miyaura route to phenyl-naphthalene macroarray **7a–b**. Conversions and purities are average values. Abbreviations: DIC *N,N'*-diisopropylcarbodiimide, DMF *N,N*-dimethylformamide, NMP = *N*-methyl-2-pyrrolidone, TFA = trifluoroacetic acid.

Fig. 2.

A) CuAAC route to 1,4-disubstitued 1,2,3-triazoles **11**. Conversions and purities are average values. Abbreviations: CuI = copper(I) iodide, Pyr = pyridine, 2-AcPyr = 2-acetylpyridine. B) Alkyne building blocks (**8**) used to construct the CuAAC macroarray. C) Azide building blocks (**10**) used to construct the CuAAC macroarray.

Fig. 3. Sequential CuAAC/Suzuki–Miyaura reaction route to a macroarray of biphenyl-triazoles (**13**). See Figs. 1 and 2 for abbreviations. Alkyne initial building block (**9-A**) loading was 110 nanomoles.

Fig. 4.

Representative *V. fischeri* LuxR screening data for the phenyl-naphthalene library **7a–b**. Data for the most active LuxR agonists identified in *V. fischeri* are shown (phenyl-naphthalenes **7b–R**, with R = functionality indicated on the *x*-axis). Luminescence activation data (1 : 1 against OHHL, 2 μ M compound). Strain: *V. fischeri* ES114 $\Delta luxI$. Neg. control = DMSO. Pos. control = 2 μ M OHHL. Error approximately $\pm 5\%$ (see ESI† for assay protocol and screening results for the full macroarray **7a–b**).

Table 1

Purity and conversion data for representative phenyl-naphthalene macroarray products 7a–b

Phenyl- naphthalene ^a	R	Conversion (%) ^b	Purity (%) ^c
7a-1	H	>98	>98
7a-4	4-Cl	>98	95.9
7a-6	3-F	>98	>98
7a-8	3-COCH ₃	>98	>98
7a-10	4-CO ₂ Et	96.6	95.0
7a-14	2-Me	>98	>98
7a-17	2-OMe	>98	>98
7a-20	3-Me, 4-OMe	>98	>98
7a-24	3-OH	>98	>98
7a-33	3-F, 4- <i>i</i> Pr	>98	96.5
7b-9	4-COCH ₃	>98	>98
7b-11	4-CF ₃	>98	>98
7b-12	4-CN	>98	>98
7b-13	3-NO ₂	97.9	96.8
7b-19	4-OMe	>98	>98
7b-25	4-OH	95.5	95.1
7b-26	4-NMe ₂	>98	97.2
7b-28	3-F, 4-Me	>98	>98
7b-32	2-F, 3-OMe	>98	>98
7b-34	3-F, 4- <i>On</i> Pr	>98	95.9

^aThe second number of the compound name (**7a-X**) corresponds to the boronic acid building block. See ESI† for full product structures for the macroarray.

^bCalculated by comparing the initial loadings of aryl bromides **4a–b** on the macroarray and the quantity remaining after the Suzuki–Miyaura reaction using calibration curves; see ESI† for full details.

^cHPLC UV detection at 254 nm; see ESI† for HPLC traces.

Table 2

Purity and conversion data for representative 1,2,3-triazole macroarray products 11

Triazole ^a	Alkyne (8)	Azide (10)	Conversion (%) ^b	Purity (%) ^c
11-A,A	A	A	>98	>98
11-A,E	A	E	>98	97.4
11-A,F	A	F	>98	>98
11-A,G	A	G	>98	>98
11-A,I	A	I	97.1	96.2
11-B,B	B	B	>98	>98
11-B,C	B	C	>98	97.8
11-B,H	B	H	>98	>98
11-B,J	B	J	>98	96.6
11-B,K	B	K	>98	97.8
11-C,C	C	C	>98	>98
11-C,D	C	D	>98	>98
11-C,F	C	F	>98	>98
11-C,I	C	I	>98	>98
11-C,J	C	J	94.8	93.2
11-D,B	D	B	>98	97.0
11-D,F	D	F	>98	>98
11-D,H	D	H	>98	96.9
11-D,I	D	I	97.3	94.3
11-D,K	D	K	>98	96.5

^aThe two letters of the compound name (**11-X,X**) correspond to the alkyne and azide building blocks, respectively. See ESI† for full product structures for the macroarray.

^bCalculated by comparing initial alkyne **8** loadings and the quantity remaining after the CuAAC reaction using calibration curves; see ESI† for full details.

^cHPLC UV detection at 254 nm; see ESI† for HPLC traces.

Table 3

Purity and conversion data for the CuAAC/Suzuki–Miyaura biphenyl-triazole macroarray (13)

CuAAC/Suzuki–Miyaura product ^a	R	Conversion [%] ^b	Purity [%] ^c
13-1	H	>98	>98
13-3	3-Cl	>98	96
13-4	4-Cl	>98	95
13-5	2-F	>98	95
13-7	4-F	>98	97
13-8	3-COCH ₃	>98	95
13-14	2-Me	>98	97
13-16	4-Me	>98	>98
13-18	3-OMe	>98	>98
13-19	4-OMe	>98	98
13-23	1,3-benzo-dioxol-5-yl ^d	>98	98
13-24	3-OH	>98	>98
13-26	4-NMe ₂	>98	98
13-29	2-Me, 5-F	>98	95
13-30	3-Cl, 4-OMe	>98	98
13-31	3-F, 4-OMe	>98	>98
13-32	2-F, 3-OMe	>98	97
13-35	6-methoxy-2-naphthyl ^d	>98	98

^aThe second number of the compound name (13-X) corresponds to the boronic acid building block. See ESI† for full product structures for the macroarray.

^bSee footnotes for Table 2.

^cSee footnotes for Table 2.

^dName of boronic acid substituent.