# Efficient Solid-Phase Synthesis of a Library of Distamycin Analogs Containing Novel Biaryl Motifs on SynPhase Lanterns

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Distamycin is a naturally occurring antibiotic that binds to AT-rich sequences in the minor groove of DNA in a noncovalent manner. It continues to be of interest as a "building block" for more-complex small-molecule ligands capable of targeting specific DNA sequences for gene regulation purposes (i.e., transcription factor inhibitors). We report here a convenient and efficient synthesis of a library of 72 novel analogs (**3a**−**f**) of the parent distamycin on SynPhase Lanterns. To investigate structure−activity relationships including DNA-binding affinity and sequence-selectivity, two previously unexplored points of diversification have been introduced into the distamycin structure by replacing one of its pyrrole rings with novel biaryl motifs. The key aspects of the synthetic approach include the development of an efficient protocol for preparation of the heterocyclic polyamide chain, optimization of the Suzuki−Miyaura cross-coupling reaction and application of a split-and-mix technique based on radiofrequency encoding. In addition, a series of biaryl carboxamide derivatives (**4a**−**f**) has been synthesized utilizing the title library diversity reagents.

## Introduction

There is much interest in synthesizing low molecular weight drug-like ligands that can bind with high affinity to discrete sequences of DNA. Such compounds have the potential to selectively block a number of DNA-related processes such as transcription and replication and, in cases where sequences of DNA not appearing in the human host genome can be clearly identified, could be developed as highly selective anticancer, antibacterial, and antiparasitic agents.[1−4] Distamycin (**1**) and netropsin (**2**) (Figure 1) are two naturally occurring compounds from *Streptomyces* species,[5,6] which have generated significant interest in the gene targeting area through the years because of their drug-like properties and their ability to recognize AT-rich sequences through binding noncovalently in the minor groove of B-form DNA.[7,8] However, despite a substantial research effort during the past 20 years, it has proved challenging to modify the structure of the distamycin framework in a rational manner to improve sequence selectivity.[9] Although there is a substantial literature on structure−activity relationships, the addition of biaryl units to the distamycin framework has not yet been explored and is the subject of this study.

The application of combinatorial chemistry using both solution- and solid-phase methodologies has proved successful in accelerating the synthesis of chemical libraries for drug discovery purposes.[10] While solution-phase combinatorial chemistry has been previously applied to the synthesis of distamycin derivatives[11] and a solid-phase method involving mass-directed preparative HPLC has been developed for the synthesis of higher homologues of distamycin,[12] to date there are few examples of the application of solid-phase combinatorial approaches to the design of novel distamycin analogs.[13] Therefore, we have developed a solid-phase combinatorial methodology based on the SynPhase radiofrequency tagging system to produce libraries of heteroaromatic polyamides to further explore the SAR of molecules of this type. In particular, it was reasoned that the novel biaryl subunits might overcome H-bond registry issues relating to polyamides containing repetitive sequences of *N*-methylpyrrole units.[14,15] Historically, this has been one of the major

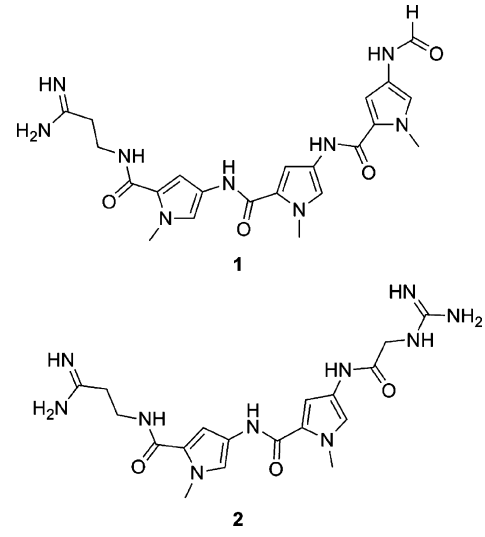


**Figure 1.** Structures of the naturally occurring DNA minor-groove binding agents distamycin (**1**) and netropsin (**2**).

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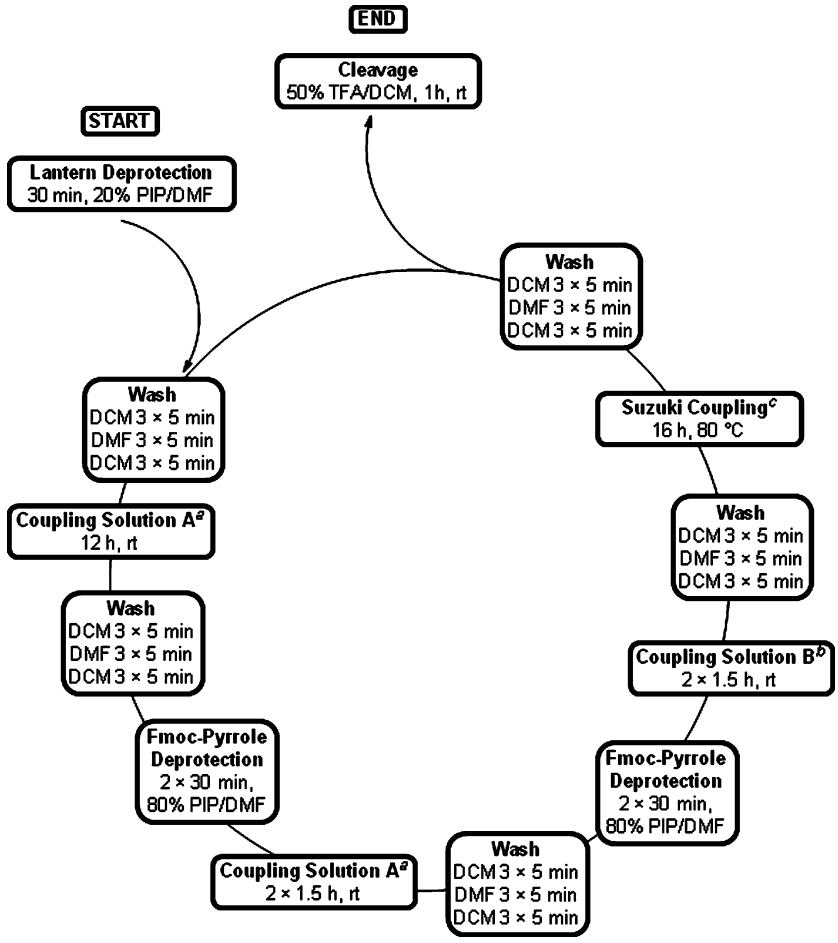
**Scheme 1.** Coupling Cycle for Preparation of Libraries **1** and **2**[a]




[a] Coupling Solutions A, B and C: (a) **10** (0.2 M), HOBt (0.2 M), DIC (0.2 M), dry DMF/DCM (50/50); (b) reagent chemset **5** (0.2 M), HOBt (0.2 M), DIC (0.2 M), dry DMF/DCM (50/50); (c) reagent chemset **6** (0.05 M), $Pd(PPh_3)_4$ (0.02 M), $Na_2CO_3$ (0.25 M), 10% $H_2O$/DMF. PIP = piperidine.

problems relating to the design of distamycin-based analogs capable of recognizing discrete DNA sequences.

The synthetic methodologies developed in this study are robust, efficient and economic and, at the end of the coupling cycle (Scheme 1) the majority of library molecules did not require further HPLC purification (see Table 4) and could be used directly in high-throughput screens such as the ethidium bromide displacement assay.[16]

## Results and Discussion

Our synthetic approach involved (1) deletion of the distamycin C-terminus amidine-containing chain (achieved through amide coupling reactions on Rink Amide Linker SynPhase Lanterns, which yielded primary carboxamides after acid cleavage), and (2) replacement of the naturally occurring N-terminus (*N*-methylpyrrole-4-formamido)-2-yl unit with novel biaryl motifs (Figure 2). The Pd-catalyzed Suzuki−Miyaura reaction of aryl halides with boronic acids was considered the best method[17] to achieve the second step which involved carbon−carbon bond formation of biaryl motifs.[18,19] Therefore, an efficient coupling cycle involving optimized protocols for assembly of the heteroaromatic polyamide chain and for the Suzuki−Miyaura cross-coupling reaction was developed (Scheme 1).

A library of 72 tetra-heteroaromatic polyamides (Figure 3, library **1**) was generated with two points of diversity at

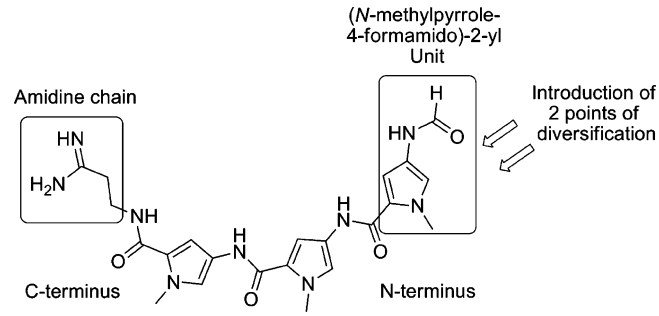



**Figure 2.** Structure of distamycin and the points of diversification introduced in the analogs.

the third and fourth positions of the library template using reagent chemsets **5** and **6** (Figure 4). For descriptive purposes the compounds were divided according to their third cyclic moiety into six sublibraries **3a**−**f**. In addition, library **2** (Figure 3) was prepared to provide the 72 biaryl carboxamide compounds necessary for library production, and whose intermediate **8** served as the substrate for the optimized Suzuki cross-coupling reaction. By analogy with compounds in libraries **1** and **2**, members were divided into six sublibraries, **4a**−**f**, according to their first cyclic moiety.

Initially, efforts were focused on optimization of the Suzuki−Miyaura cross-coupling reaction and the synthesis of **4**{*1*,*1*} (Scheme 2). Quantitative coupling of reagent **5**{*1*} onto deprotected Rink-amino Lanterns (**7**) was achieved in

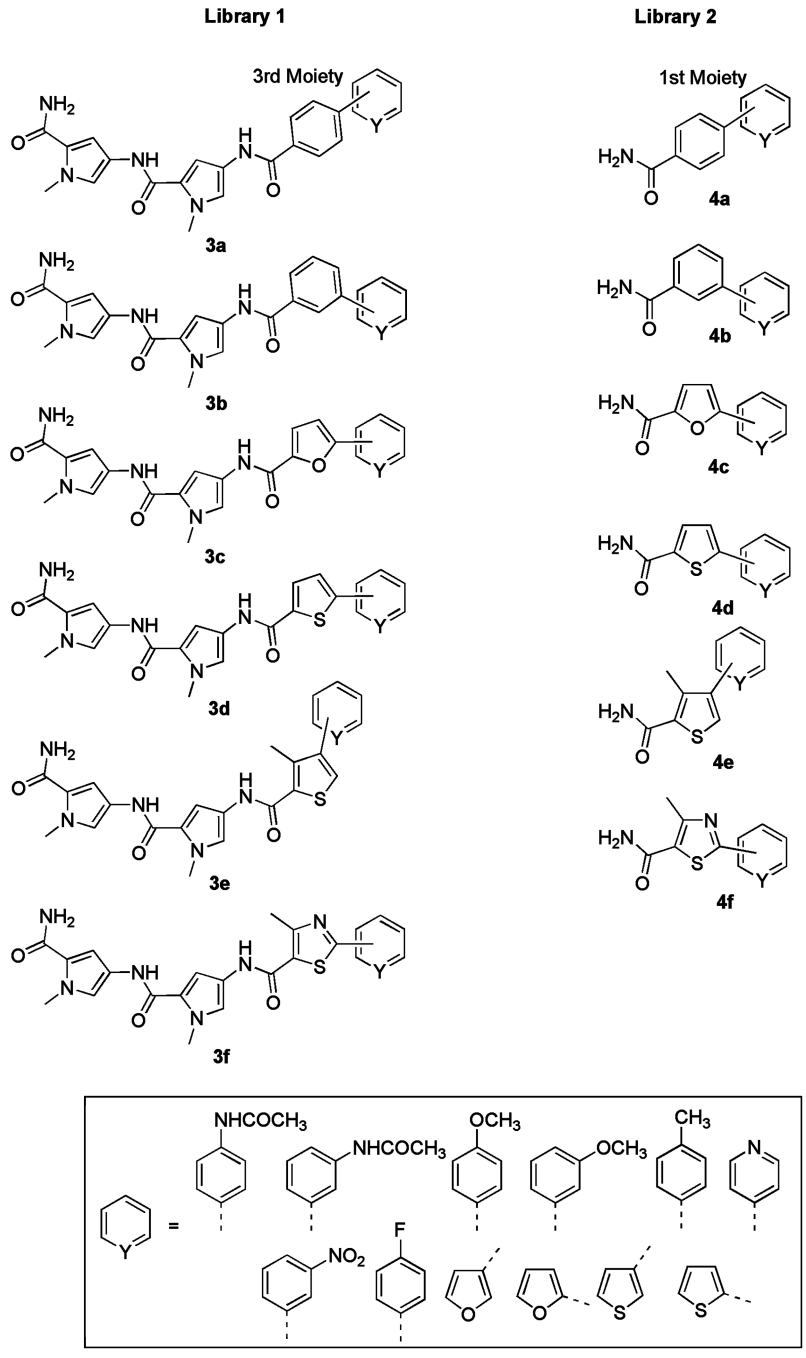



**Figure 3.** Structures of libraries **1** and **2** templates.

12 h with HOBt−DIC (0.2 M) to yield the polymer-bound bromoderivative **8**{*1*}.[20] The loading was determined by cleaving **8**{*1*} from one lantern (50% TFA/DCM, 1 h), and the resulting product **9**{*1*} was weighed and analyzed by LC-MS.

Optimization of the Suzuki cross-coupling reaction of **8**{*1*} with 1.4 equiv of reagent **6**{*1*} was achieved using two catalytic systems, $Pd(PPh_3)_4$ and $Pd(dba)_2/P(o\text{-}tol)_3$ at four different concentrations (0.005, 0.01, 0.015, and 0.02 M), two bases, $Na_2CO_3$ (0.25 M) and $Et_3N$ (0.35 M), and two solvent mixtures (toluene/EtOH/$H_2O$:1/1/0.1 and DMF/$H_2O$: 9/1) to give a total of 32 separate experiments. The reactions were heated at 80 °C for the designated time, then cooled, washed, and cleaved with 50% TFA/DCM to produce **4**{*1*,*1*}. The crude samples were weighed and analyzed by analytical HPLC. Table 1 illustrates the most significant results, with $Pd(PPh_3)_4$ proving to be the best catalyst. The chemistry of $P(o\text{-}tol_3)$ as the supporting ligand for in situ formation of the active Pd complex was inefficient. Initial attempts to utilize chelating phosphine catalysts, such as $PdCl_2$(dppf), did not give satisfactory results with traces of the ligand evident in final samples,[21] and so this chemistry was not investigated any further. The presence of unreacted bromoheteroaromatic carboxamide **8** in the cleavage solutions of experiments **3**−**5** suggested that a stronger base than $Et_3N$ was required, and replacement with $Na_2CO_3$ resulted in a more efficient reaction. Finally, the observation that cross-coupling reactions proceeded at higher rates when using DMF/$H_2O$ as solvent enabled optimization of the synthesis of **4**{*1*,*1*} (see experiment 9, Table 1).

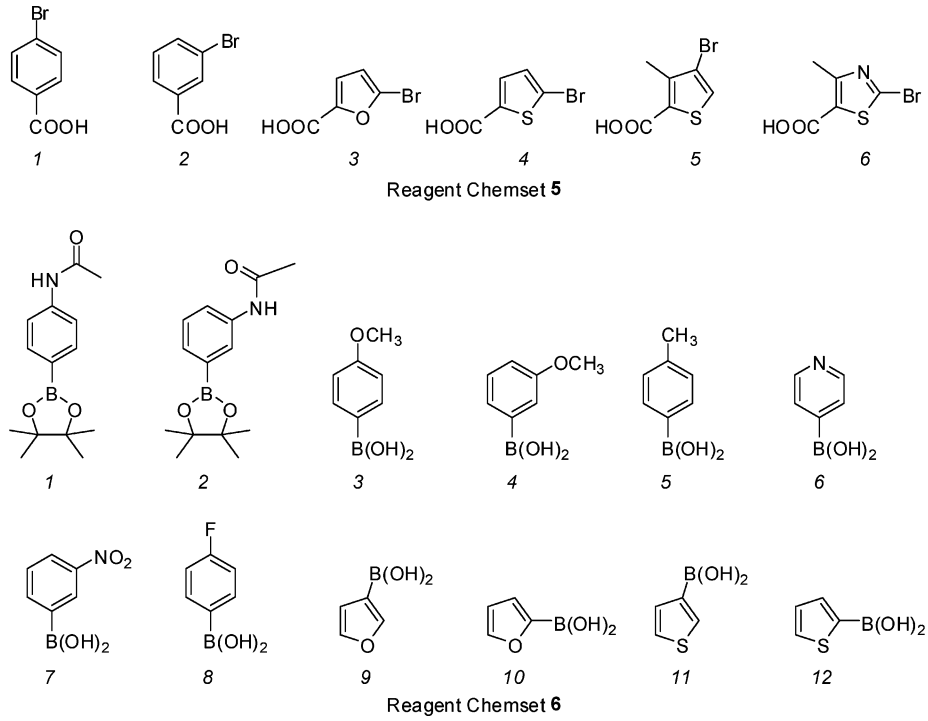
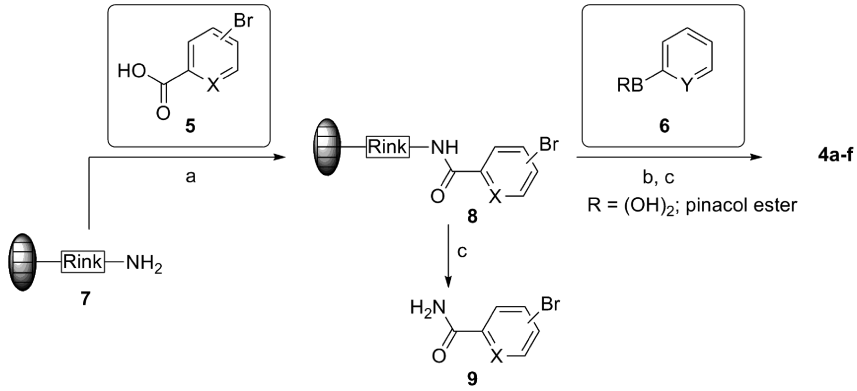



**Figure 4.** Structures of reagent chemsets **5** and **6**.

**Scheme 2.** Reaction Scheme for the Synthesis of Biaryl Carboxamide Derivatives **4a**−**f**[a]




[a] Reaction Conditions: (a) reagent chemset **5** (carboxylic acids) (0.2 M), HOBt (0.2 M), DIC (0.2 M), dry DMF/DCM (50/50), 12 h; reagent chemset **6** (boronic acid/esters) (0.5 M), $Pd(PPh_3)_4$ (0.02 M), $Na_2CO_3$ (0.25 M), DMF/$H_2O$ (90/10), 80 °C, 16 h; (c) 50% TFA/DCM, 1 h.

**Table 1.** Optimization of Suzuki Cross-Coupling Reaction for the Synthesis of **4**{*1*,*1*}

| experiment | catalytic system (M) | base (M) | solvent | reaction time (h) | yield[a] (%) | purity[b] (%) |
|---|---|---|---|---|---|---|
| 1 | $Pd(PPh_3)_4$ (0.005) | $Na_2CO_3$ (0.25) | toluene/EtOH/$H_2O$ 1/1/0.1 | 48 | 30 | 55 |
| 2 | $Pd(PPh_3)_4$ (0.01) | $Na_2CO_3$ (0.25) | toluene/EtOH/$H_2O$ 1/1/0.1 | 24 | 40 | 60 |
| 3 | $Pd(PPh_3)_4$ (0.01) | $Et_3N$ (0.35) | toluene/EtOH/$H_2O$ 1/1/0.1 | 32 | 40 | 65 |
| 4 | $Pd(PPh_3)_4$ (0.01) | $Et_3N$ (0.35) | DMF/$H_2O$ 9/1 | 16 | 45 | 75 |
| 5 | $Pd(PPh_3)_4$ (0.015) | $Et_3N$ (0.35) | DMF/$H_2O$ 9/1 | 16 | 70 | 85 |
| 6 | $Pd(PPh_3)_4$ (0.01) | $Na_2CO_3$ (0.25) | DMF/$H_2O$ 9/1 | 16 | 50 | 75 |
| 7 | $Pd(PPh_3)_4$ (0.015) | $Na_2CO_3$ (0.25) | DMF/$H_2O$ 9/1 | 16 | 75 | 90 |
| 8 | $Pd(PPh_3)_4$ (0.01) | $Na_2CO_3$ (0.25) | DMF/$H_2O$ 9/1 | 16 | 85 | 90 |
| 9 | $Pd(PPh_3)_4$ (0.02) | $Na_2CO_3$ (0.25) | DMF/$H_2O$ 9/1 | 16 | >99 | >99 |
| 10 | $Pd(dba)_2$ (0.015)/ P(*o*-tol)$_3$ (0.15) | $Na_2CO_3$ (0.25) | toluene/EtOH/$H_2O$ 1/1/0.1 | 46 | 50 | 40 |
| 11 | $Pd(dba)_2$ (0.02)/ P(*o*-tol)$_3$ (0.02) | $Na_2CO_3$ (0.25) | DMF/$H_2O$ 9/1 | 16 | 50 | 42 |

[a] Yields were based on recovery and were relative to the initial loading of the lanterns. [b] The purity of the crude material was determined using reverse-phase HPLC with UV detection (250 nm).

An exploratory library (library **2**) was then synthesized in a combinatorial manner using the split-and-mix technique[22] to assess the scope and limitations of the optimized Suzuki cross-coupling method. Members of reagent chemset **5**{*1*−*6*} were quantitatively loaded onto radiofrequency tagged lanterns (**7**) to yield the lantern-bound bromo-derivatives **8**{*1*−*6*}, which were in turn cross-coupled with members of chemset **6**{*1*−*12*} using the optimized Suzuki reaction conditions to give, after release from the Lanterns, **4a**−**f** (library **2**, Table 2). All products were analyzed by analytical

**Table 2.** Purity, HPLC Data, and Yields for Library 2 Members

| entry | **4a** | purity[a] (%) | RT (min) | yield[b] (%) | entry | **4b** | purity[a] (%) | RT (min) | yield[b] (%) | entry | **4c** | purity[a] (%) | RT (min) | yield[b] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | {*1,1*} | 99 | 4.45 | 99 | 13 | {*2,1*} | 90 | 4.58 | 90 | 25 | {*3,1*} | 80 | 4.45 | 79 |
| 2 | {*1,2*} | 87 | 4.58 | 90 | 14 | {*2,2*} | 82 | 4.68 | 60 | 26 | {*3,2*} | 97 | 4.38 | 99 |
| 3 | {*1,3*} | 94 | 5.37 | 90 | 15 | {*2,3*} | 72 | 5.38 | 72 | 27 | {*3,3*} | 98 | 5.05 | 99 |
| 4 | {*1,4*} | 99 | 5.40 | 99 | 16 | {*2,4*} | 95 | 5.37 | 99 | 28 | {*3,4*} | 99 | 5.17 | 99 |
| 5 | {*1,5*} | 84 | 5.71 | 90 | 17 | {*2,5*} | 97 | 5.71 | 99 | 29 | {*3,5*} | 99 | 5.43 | 99 |
| 6 | {*1,6*} | 50 | 5.64 | 40 | 18 | {*2,6*} | 43 | 5.60 | 40 | 30 | {*3,6*} | 40 | 5.65 | 35 |
| 7 | {*1,7*} | 82 | 5.35 | 76 | 19 | {*2,7*} | 98 | 5.32 | 99 | 31 | {*3,7*} | 99 | 5.08 | 99 |
| 8 | {*1,8*} | 92 | 5.50 | 90 | 20 | {*2,8*} | 80 | 5.48 | 75 | 32 | {*3,8*} | 99 | 5.10 | 99 |
| 9 | {*1,9*} | 58 | 4.47 | 70 | 21 | {*2,9*} | 57 | 4.48 | 50 | 33 | {*3,9*} | 84 | 4.35 | 64 |
| 10 | {*1,10*} | 41 | 4.47 | 41 | 22 | {*2,10*} | 86 | 4.38 | 40 | 34 | {*3,10*} | 85 | 4.85 | 50 |
| 11 | {*1,11*} | 88 | 5.17 | 90 | 23 | {*2,11*} | 96 | 5.18 | 93 | 35 | {*3,11*} | 99 | 4.82 | 99 |
| 12 | {*1,12*} | 81 | 4.47 | 59 | 24 | {*2,12*} | 39 | 4.37 | 35 | 36 | {*3,12*} | 82 | 4.66 | 40 |

| entry | **4d** | purity[a,c] (%) | RT (min)[c] | yield[b,c] (%) | entry | **4e** | purity[a,c] (%) | RT (min)[c] | yield[b,c] (%) | entry | **4f** | purity[a,c] (%) | RT (min)[c] | yield[b,c] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 37 | {*4,1*} | 99 | 4.52 | 99 | 49 | {*5,1*} | 99 | 4.63 | 99 | 61 | {*6,1*} | 86 | 4.40 | 50 |
| 38 | {*4,2*} | 99 | 4.68 | 99 | 50 | {*5,2*} | 99 | 4.55 | 99 | 62 | {*6,2*} | 83 | 4.42 | 50 |
| 39 | {*4,3*} | 95 | 5.50 | 93 | 51 | {*5,3*} | 99 | 5.37 | 99 | 63 | {*6,3*} | 80 | 5.08 | 77 |
| 40 | {*4,4*} | 96 | 5.52 | 92 | 52 | {*5,4*} | 99 | 5.40 | 99 | 64 | {*6,4*} | 83 | 5.15 | 83 |
| 41 | {*4,5*} | 99 | 5.91 | 99 | 53 | {*5,5*} | 94 | 5.73 | 92 | 65 | {*6,5*} | 80 | 5.42 | 77 |
| 42 | {*4,6*} | NR | NR | NR | 54 | {*5,6*} | NR | NR | NR | 66 | {*6,6*} | 40 | 5.66 | 35 |
| 43 | {*4,7*} | 92 | 5.43 | 90 | 55 | {*5,7*} | 99 | 5.33 | 99 | 67 | {*6,7*} | 65 | 5.18 | 45 |
| 44 | {*4,8*} | 99 | 5.60 | 99 | 56 | {*5,8*} | 96 | 5.45 | 93 | 68 | {*6,8*} | 82 | 5.17 | 60 |
| 45 | {*4,9*} | 95 | 4.72 | 60 | 57 | {*5,9*} | 36 | 4.32 | 32 | 69 | {*6,9*} | NR | NR | NR |
| 46 | {*4,10*} | 94 | 4.72 | 64 | 58 | {*5,10*} | 83 | 4.33 | 62 | 70 | {*6,10*} | NR | NR | NR |
| 47 | {*4,11*} | 84 | 5.33 | 60 | 59 | {*5,11*} | 89 | 5.12 | 86 | 71 | {*6,11*} | 22 | 4.67 | 18 |
| 48 | {*4,12*} | NR | NR | NR | 60 | {*5,12*} | 99 | 4.32 | 99 | 72 | {*6,12*} | NR | NR | NR |

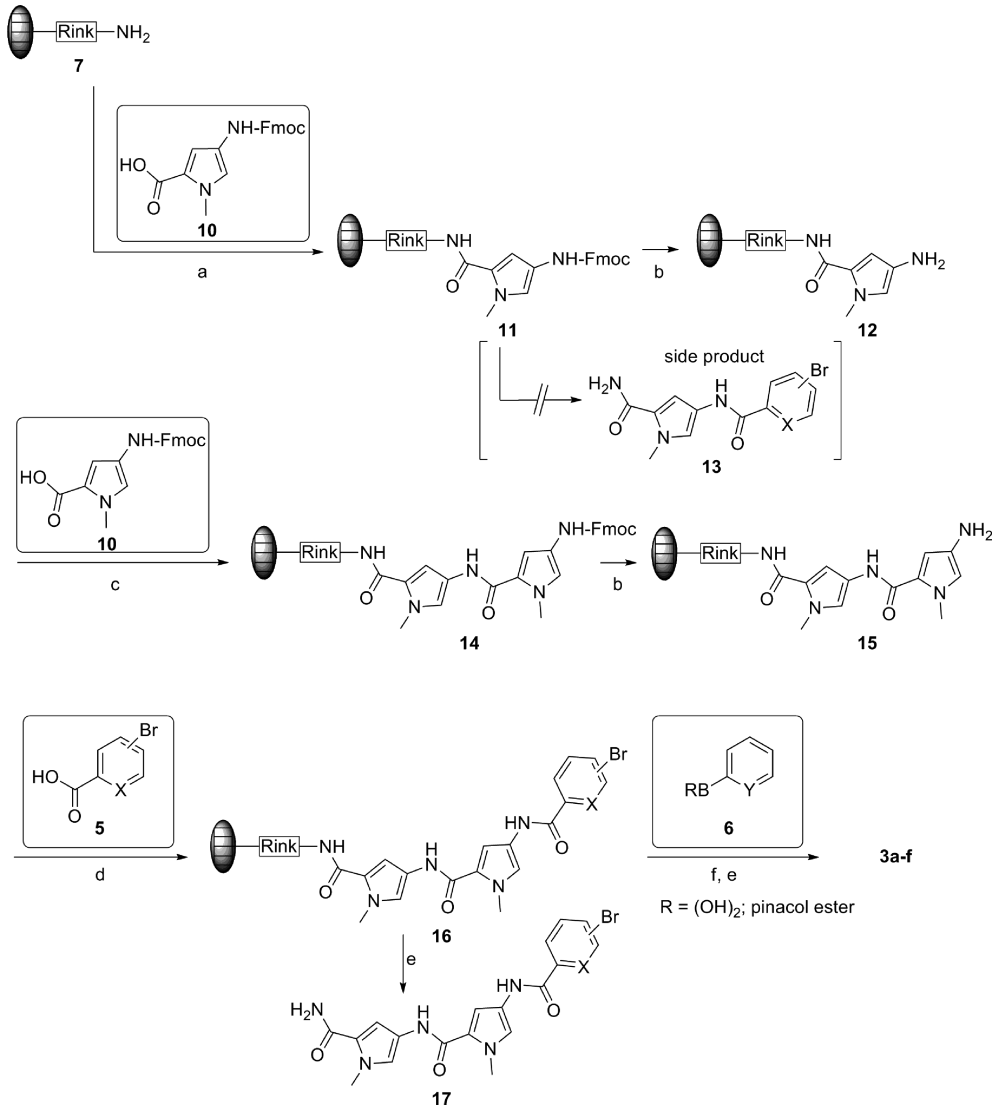
[a] The purity of the crude material was determined using reversed-phase HPLC with UV detection (250 nm). [b] Yield based on recovery and purity. [c] NR = No reaction.

HPLC (UV, 250 nm) and the yields calculated with respect to the initial loading of the lantern. The excellent overall yield and purity of library members **4a**−**f** showed that the Suzuki-optimized conditions were suitable for a wide variety of lantern-bound aryl/heteroaryl halides **8**{*1*−*6*} and boronic acid/esters **6**{*1*−*12*}. However, compounds **4**{*1*−*6,6*} were an exception as members of this subset were produced in very low yield or not at all. This was possibly due to the 4-pyridineboronic acid **6**{*6*} forming a catalytically inactive pyridyl-palladium complex.[23] Conversely, the yield and purity observed for sublibraries **4c**, **4d**, and **4e** were the highest of all the sublibraries, suggesting that lantern-bound 5-bromofuran **8**{*3*}, 5-bromothiophene **8**{*4*}, and 4-bromo-3-methylthiophene **8**{*5*} are better substrates for Suzuki cross-coupling reactions in comparison to the other lantern-bound bromo-substituted derivatives examined. In these cases, the high electrophilicity of 5-halo-substituted furans and 5-halo/4-halo-3-methyl-substituted thiophenes may favor the rate determining oxidative addition step in the Pd(0) catalytic cycle.[24]

After optimization of the Suzuki cross-coupling methodology, the focus changed to development of the amide coupling protocol necessary for synthesis of the polymer-bound bromo-triheterocycle polyamides **16**{*1*−*6*} (Scheme 3). The latter were required as substrates for the Suzuki cross-coupling reactions with **6**{*1*−*12*} to afford the title tetraheterocyclic polyamides **3a**−**f**, diversified at their third and fourth positions through the use of split-and-mix technology. The coupling of Fmoc-protected 4-aminopyrrole-2-carboxylic acid (**10**) to **12** to produce the lantern-bound amino-dipyrrole platform **15**, followed by subsequent coupling with members of chemset **5**{*1*−*6*} to afford the polymer-bound bromo derivatives **16**{*1*−*6*}, initially posed a complex challenge. Preliminary amide coupling experiments to synthesize one of the title polyamides in good yield and purity were initially unsuccessful. During the polyamide chain assembly, insufficient deprotection of lantern-bound Fmoc-protected derivatives **11** and **14** occurred, along with incomplete amide coupling of carboxylic acids **10** and **5**{*1*−*6*} to lantern-bound unprotected amino derivatives **12** and **15**, respectively. In addition, after acid cleavage, unwanted polyamide oligomers of different chain lengths were obtained. In particular, formation of **13** was prevalent, indicating that crucial steps for optimization were the Fmoc deprotection of polymer-bound residue **11**, the coupling of **10** with lantern-bound amino derivative **12**, and the Fmoc-deprotection of **14** to form **15**. The assembly of **14** and its deprotection to provide **15** were investigated first, with coupling efficiencies for each step analyzed using an UV-based quantitative Fmoc assay.[25−27] After carrying out a number of experiments in which reaction conditions were gradually improved, it was discovered that while the first pyrrole residue could be loaded onto the lantern (**7**) with 95% efficiency after 12 h (experiment 3, Table 3), attachment of the second pyrrole residue reached 95% yield only after two additions of fresh coupling solution (A) to **12** followed by two deprotection cycles of **14** with deprotection solution (C) (experiment 6, Table 3).

This optimized approach was used to achieve quantitative loading of the amino-dipyrrole platform **15** onto lantern **7**, and the subsequent amide coupling reaction with **5**{*1*−*6*} to form the Suzuki cross-coupling substrate **16**{*1*−*6*} then attempted. The coupling efficiency of this step was measured by loading **5**{*1*−*1*} onto the lantern-bound amino-dipyrrole platform **15**, followed by acid cleavage (50% TFA/DCM, 1 h) of **16**{*1*−*1*} from the polymer support followed by both quantitative and

**Scheme 3.** Reaction Scheme for the Synthesis of Tetra-Heteroaromatic Polyamides **3a**−**f**[a]




[a] Reaction conditions: (a) **10** (0.2 M), HOBt (0.2 M), DIC (0.2 M), dry DMF/DCM (50/50), 12 h; (b) 80% PIP/DMF, 30 min (×2); (c) **10** (0.2 M), HOBt (0.2 M), DIC (0.2 M), dry DMF/DCM (50/50), 1.5 h (×2); (d) reagent chemset **5** (carboxylic acids) (0.2 M), HOBt (0.2 M), DIC (0.2 M), dry DMF/DCM (50/50), 1.5 h, room temperature (×2); (e) 50% TFA/DCM, 1 h; (f) reagent chemset **6** (boronic acid/esters) (0.5 M), $Pd(PPh_3)_4$ (0.02 M), $Na_2CO_3$ (0.25 M), DMF/$H_2O$ (90/10), 80 °C, 16 h.

**Table 3.** Optimization of Lantern-Bound Amino-dipyrrole Platform **15** and Bromo-polyamide **17**{*1*−*1*} Assembly

| | amino-dipyrrole platform **15** | | | | | | bromo-polyamide **17**{*1*−*1*} | | |
|---|---|---|---|---|---|---|---|---|---|
| | first pyrrole residue | | | second pyrrole residue | | | **5**{*1*−*1*} | | |
| experiments | coupling solution A[a] time (repeat) | deprotection solution C[b] time (repeat) | % Fmoc released[c] | coupling solution A[a] time (repeat) | deprotection solution C[b] time (repeat) | % Fmoc released[c] | coupling solution B[d] time (repeat) | % area[e] for **13** (RT = 5.34) | % area[e] for **17** (RT = 5.52) |
| 1 | 1.5 h (×1) | 30 min (×1) | 45 | | | | | | |
| 2 | 12 h (×1) | 30 min (×1) | 75 | | | | | | |
| 3 | 12 h (×1) | 30 min (×2) | **95** | 1.5 h (×1) | 30 min (×1) | 60 | | | |
| 4 | 12 h (×1) | 30 min (×2) | 95 | 1.5 h (×2) | 30 min (×1) | 80 | | | |
| 6 | 12 h (×1) | 30 min (×2) | 95 | 1.5 h (×2) | 30 min (×2) | **95** | 1.5 h (×1) | 35 | 55 |
| 7 | 12 h (×1) | 30 min (×2) | 95 | 1.5 h (×2) | 30 min (×2) | 95 | 1.5 h (×2) | <5 | **95** |

[a] 4-(9H-Fluoren-9-ylmethoxycarbonylamino)-1-methyl-1H-pyrrole-2-carboxylic acid **10**, HOBt-DIC (0.2 M), room temperature. [b] 80% PIP/DMF, room temperature. [c] Absorbance for quantitative Fmoc analysis was read at 301 nm (see Experimental Section). [d] Reagent chemset **5**{*1*−*6*} (0.2 M), HOBt−DIC (0.2 M), room temperature. [e] The purity of the crude material was determined using reversed-phase HPLC with UV detection (250 nm).

LC-MS analysis of the released bromo-polyamide **17**{*1*−*1*}. After several experiments, it was established that two consecutive additions of coupling solution (B) were required for formation of **17**{*1*−*1*} in high purity (experiment 7, Table 3). This allowed a standard amide coupling protocol to be optimized for formation of lantern-bound bromo-heterocycle polyamides of type **16**{*1*−*6*} which were then cross-coupled with substrates **6**{*1*−*12*} using the Suzuki reaction conditions described above (experiment 9, Table 1) to afford library members **3a**−**f** (Table 4).

**Table 4.** Purity and HPLC Data for Library **1** Members

| entry | **3a** | purity (%)[a,b] | RT (min)[b] | entry | **3b** | purity (%)[a,b] | RT (min)[b] | entry | **3c** | purity (%)[a] | RT (min) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | {*1*,*1*} | 80 | 5.25 | 13 | {*2*,*1*} | 90 | 5.38 | 25 | {*3*,*1*} | 90 | 5.20 |
| 2 | {*1*,*2*} | 87 | 5.32 | 14 | {*2*,*2*} | 82 | 5.38 | 26 | {*3*,*2*} | 99 | 5.25 |
| 3 | {*1*,*3*} | 95 | 5.97 | 15 | {*2*,*3*} | 80 | 5.98 | 27 | {*3*,*3*} | 99 | 5.90 |
| 4 | {*1*,*4*} | 80 | 5.90 | 16 | {*2*,*4*} | 92 | 5.92 | 28 | {*3*,*4*} | 92 | 5.97 |
| 5 | {*1*,*5*} | 63 | 6.15 | 17 | {*2*,*5*} | 99 | 6.15 | 29 | {*3*,*5*} | 99 | 6.22 |
| 6 | {*1*,*6*} | NR | NR | 18 | {*2*,*6*} | NR | NR | 30 | {*3*,*6*} | 39 | 5.88 |
| 7 | {*1*,*7*} | 90 | 5.35 | 19 | {*2*,*7*} | 89 | 5.92 | 31 | {*3*,*7*} | 99 | 5.08 |
| 8 | {*1*,*8*} | 92 | 5.50 | 20 | {*2*,*8*} | 80 | 5.48 | 32 | {*3*,*8*} | 99 | 5.45 |
| 9 | {*1*,*9*} | 85 | 5.58 | 21 | {*2*,*9*} | 57 | 5.48 | 33 | {*3*,*9*} | 84 | 5.45 |
| 10 | {*1*,*10*} | 39 | 5.47 | 22 | {*2*,*10*} | 42 | 5.38 | 34 | {*3*,*10*} | 85 | 5.45 |
| 11 | {*1*,*11*} | 87 | 5.57 | 23 | {*2*,*11*} | 96 | 5.58 | 35 | {*3*,*11*} | 95 | 5.55 |
| 12 | {*1*,*12*} | 55 | 5.51 | 24 | {*2*,*12*} | 39 | 5.47 | 36 | {*3*,*12*} | 42 | 5.66 |

| entry | **3d** | purity (%)[a,b] | RT (min)[b] | entry | **3e** | purity (%)[a,b] | RT (min) | entry | **3f** | purity (%)[a,b] | RT (min)[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 37 | {*4*,*1*} | 99 | 5.33 | 49 | {*5*,*1*} | 99 | 5.32 | 61 | {*6*,*1*} | 96 | 5.15 |
| 38 | {*4*,*2*} | 95 | 5.33 | 50 | {*5*,*2*} | 93 | 5.33 | 62 | {*6*,*2*} | 94 | 4.47 |
| 39 | {*4*,*3*} | 80 | 5.90 | 51 | {*5*,*3*} | 99 | 5.97 | 63 | {*6*,*3*} | 81 | 5.98 |
| 40 | {*4*,*4*} | 96 | 5.92 | 52 | {*5*,*4*} | 99 | 5.90 | 64 | {*6*,*4*} | 84 | 5.95 |
| 41 | {*4*,*5*} | 99 | 5.90 | 53 | {*5*,*5*} | 94 | 5.73 | 65 | {*6*,*5*} | 80 | 5.72 |
| 42 | {*4*,*6*} | NR | NR | 54 | {*5*,*6*} | NR | NR | 66 | {*6*,*6*} | 45 | 5.78 |
| 43 | {*4*,*7*} | 92 | 5.93 | 55 | {*5*,*7*} | 99 | 5.93 | 67 | {*6*,*7*} | 80 | 5.98 |
| 44 | {*4*,*8*} | 99 | 5.60 | 56 | {*5*,*8*} | 96 | 5.65 | 68 | {*6*,*8*} | 85 | 5.67 |
| 45 | {*4*,*9*} | 85 | 5.73 | 57 | {*5*,*9*} | 86 | 5.72 | 69 | {*6*,*9*} | NR | NR |
| 46 | {*4*,*10*} | 86 | 5.74 | 58 | {*5*,*10*} | 83 | 5.73 | 70 | {*6*,*10*} | NR | NR |
| 47 | {*4*,*11*} | 64 | 5.33 | 59 | {*5*,*11*} | 89 | 5.12 | 71 | {*6*,*11*} | 30 | 5.67 |
| 48 | {*4*,*12*} | NR | NR | 60 | {*5*,*12*} | 99 | 4.32 | 72 | {*6*,*12*} | NR | NR |

[a] The purity of the crude material was determined using reversed-phase HPLC with UV detection (250 nm). [b] NR = No reaction.

The results shown in Table 4 indicate that the coupling cycle depicted in Scheme 1 proved to be efficient for the synthesis of tetra-heterocycle polyamides **3a**−**f**, and was tolerant of several different building blocks (**10**, Reagents **5** and **6**). As observed for library **2**, sublibraries **3c**, **3d**, and **3e** were formed with the highest purity compared to the other sublibraries, and compounds **3**{*1*−*6*,*6*} were formed in very low yield or not at all. Surprisingly, the purities of sublibrary **3f** compounds were higher compared to their sublibrary **4f** analogs.

A selection of compounds was screened in a FRET-based DNA thermal denaturation assay using a fluorescent-tagged hairpin DNA oligonucleotide (FAM-5′-TAT-AGA-TATA-TATA-TTT-TTT-TATA-TATA-TCT-ATA-3′-TAMRA) along with distamycin as a control. The most active compounds were found to have $\Delta T_m$ values approaching approximately one-third of that for distamycin under identical conditions, and experiments are ongoing to study registry issues through techniques such as molecular modeling, footprinting, X-ray crystallography and NMR.

## Conclusions

A 72-member library of distamycin analogs (**3a**−**f**) has been synthesized with two points of diversification at the third and fourth positions of the library template using a combinatorial radiofrequency tagged split-and-mix methodology. An efficient coupling-cycle protocol on polymer support was developed based on standard methods for both assembly of the heteroaromatic polyamide chain and for the Suzuki−Miyaura cross-coupling reaction. This allowed library members to be synthesized rapidly, in good yield and with acceptable purity levels without the necessity for further chromatographic purification. The broad applicability of this approach is presently being exemplified by using it to prepare combinatorial libraries of further generations of distamycin analogs with more-extensive structural modifications up to and including replacement of all constituent pyrrole rings with other well-established DNA sequence-reading heteroaromatic building-blocks. These results, along with complete DNA-interaction screening data for compounds in libraries **1** and **2** will be published elsewhere.

## Experimental Section

HPLC analyses were carried out on a Phenomenex Monolithic $C_{18}$ reversed-phase column (50 × 4.6 mm) with a flow rate of 1.5 mL $min^{-1}$ and a linear gradient of B (5−95%) over 10 min. (Eluent A: $H_2O$/0.1% formic acid; eluent B: $CH_3CN$/0.1% formic acid). Peak areas were monitored and integrated by UV (250 nm). The LC/MS system consisted of a Waters Alliance 2695 HPLC coupled to a Micromass ZQ mass spectrometer using positive electrospray ionization mode ($ES^+$). $^1H$ NMR were acquired using a Bruker Avance 400 MHz spectrometer and a Bruker Avance 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) with the solvent resonance as the internal standard (DMSO-$d_6$, $\delta$ 2.50) and coupling constants ($J$) quoted in Hertz (Hz). Spin multiplicities are described as: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), and m (multiplet). Data are reported as follows: chemical shift, multiplicity, coupling constant, integration, and assignment. Quantitative Fmoc analysis absorbance values were recorded on a Libra S22 Spectrophotometer Biochrom Ltd. All chemicals, except for 4-(9H-fluoren-9-ylmethoxycarbonylamino)-1-methyl-1*H*-pyrrole-2-carboxylic acid, which was obtained from Onyx Scientific (batch CG137H), were purchased from Aldrich and used without further purification. Fmoc-protected D-series Rink Amide Linker (RAM) SynPhase Lanterns (batch 1770−2197),

the TranSort software and TranStems were purchased from Mimotopes, Pty, Clayton, Australia. To guarantee a working volume of 0.5 mL/Lantern, all deprotection, washing and amide coupling steps were carried out in polypropylene Alltech Columns of different capacity (1.5, 4.0, 8.0, and 75 mL) and able to accommodate varying numbers of Lanterns depending on the stage of the split-and-mix campaign (see Supporting Information).

**Standard Washing Procedure.** After each deprotection and coupling step the Lanterns were sequentially washed with DCM (3 × 5 min), DMF (3 × 5 min) and DCM (3 × 5 min), and then dried in vacuo for 5 min.

**Standard Deprotection Procedure of Fmoc-Protected RAM Lanterns.** The Lanterns (72, nominal loading 36 $\mu$mol) were tagged with radiofrequency (RF) transponders and immersed in a deprotection solution of 20% piperidine (PIP)/anhydrous DMF (36 mL), shaken for 30 min at rt, and washed according to the standard procedure to afford the Fmoc-deprotected Lanterns **7**.

**Standard Procedure for Quantitative Fmoc Analysis by UV Spectrophotometry.** Each Lantern was placed in a 2.0 mL vial and treated with 1.0 mL of a solution of 2% 1,8-diazabicyclo[7]undec-7-ene (DBU) in DMF. The tube was allowed to agitate on a rotatory shaker (600−650 rpm) for 30 min at rt to promote the liberation of chromophore. At the end of this time, aliquots of 50 $\mu$L of the sample and of a reference (consisting of 2% of DBU in DMF) were both diluted to 1.0 mL with DMF in a volumetric flask by micropipettor (DF = dilution factor = 20). The two dilutions were transferred to a matched pair of 1 cm quartz glass cuvettes and the absorbance of each sample read at 301 nm and determined against the reference solution. The loading was calculated from the following equation:

$$\text{loading} = [(A_{301}/\varepsilon) \times \text{DF} \times 10^4]\ \mu\text{mol/Lantern}$$

where $A_{301}$ is the absorbance at 301 nm, $\varepsilon$ is molar extinction coefficient (9254 $M^{-1}$ $cm^{-1}$) for DBU, and DF = 20.

**Standard Procedure for the Synthesis of Lantern-Bound Amino-Pyrrole 12 Using Coupling Solution A.** Lanterns **7** (72) were immersed in coupling solution A (36 mL): **10** (0.2 M), 1-hydroxybenzotriazole (HOBt) (0.2 M), and *N*,*N*′-diisopropylcarbodiimide (DIC) (0.2 M) in 50% DCM/anhydrous DMF. The Lanterns were allowed to shake overnight at rt and washed according to the standard procedure. After the loading of **10** was determined by quantitative Fmoc analysis, the Lanterns were deprotected twice with a 80% (PIP)/anhydrous DMF solution (36 mL) for 30 min and then washed using the standard method to yield lanterns **12**.

**Standard Procedure for the Synthesis of Lantern-Bound Amino Dipyrrole Platform 15.** The Lanterns **12** (72) were immersed in coupling solution A (36 mL) and allowed to shake for a 1.5 h at rt. At this point, the reaction vessel was drained and fresh coupling solution A (36 mL) was added. The Lanterns were allowed to shake for a further 1.5 h at rt and washed according to the standard procedure. After the loading of the second residue of **10** was determined by quantitative Fmoc analysis, the Lanterns were deprotected twice with a 80% (PIP)/anhydrous DMF solution (36 mL) for 30 min and then washed using the standard method to provide lantern-bound amino dipyrrole platform **15**.

**Standard Procedure for Amide Coupling of Lantern-Bound Amino Dipyrrole Platform 15 with Reagent Chemset 5 Using Coupling Solution B.** The Lanterns **15** were divided into six fractions and immersed in six coupling solutions B (6 mL): the appropriate bromo-aryl/hetaryl carboxylic acid **5**{*1*−*6*} (0.2 M), HOBt (0.2 M), and DIC (0.2 M) in 50% DCM/anhydrous DMF, and allowed to shake for 1.5 h at rt. At this point, the reaction vessels were drained and fresh coupling solutions B added. The Lanterns were agitated for a further 1.5 h and washed according to the standard procedure to provide the lantern-bound bromo-derivatives **16**{*1*−*6*}.

**Standard Procedure for Suzuki−Miyaura Cross-Coupling Reaction of Lanterns 16 with Reagent Chemset 6.** Lanterns **16**{*1*−*6*} were divided into twelve fractions of six lanterns each, placed in sealable vials under a nitrogen atmosphere and treated with the appropriate boronic acid/esters **6**{*1*−*12*} (0.05 M), $Pd(PPh_3)_4$ (0.02 M), and $Na_2CO_3$ (0.25 M) in a 10% $H_2O$/DMF solution (3 mL) and agitated for 16 h at 80 °C. The Lanterns were washed following the standard procedure.

**Standard Procedure for TFA Cleavage.** Each lantern was placed in an Alltech column (1.5 mL) and treated with a cleavage solution of 50% TFA/DCM (0.5 mL) for 1 h at rt. After each lantern was removed from the column, the solution containing the cleaved product was transferred to a vial and concentrated using a centrifugal evaporator. The residues were lyophilized twice from 50% $CH_3CN/H_2O$ to afford the final compounds **3a**−**f**, which were analyzed by LC-MS.

**Standard Procedure for the Synthesis of Library 2 Compounds.** Lanterns **7** were coupled with the appropriate bromo-aryl/hetaryl carboxylic acid **5**{*1*−*6*} (0.2 M) using HOBt (0.2 M) and DIC (0.2 M) in 50% DCM/anhydrous DMF for 12 h at rt and washed according to the standard procedure to yield the lantern-bound bromo-derivatives **8**{*1*−*6*}. The latter were treated with the appropriate boronic acid/esters **6**{*1*−*12*} (0.05 M), $Pd(PPh_3)_4$ (0.02 M) and $Na_2CO_3$ (0.25 M) in a 10% $H_2O$/DMF solution (0.5 mL), agitated for 16 h at 80 °C, and washed following the standard procedure to provide compounds **4a**−**f**.

**Selected Spectral Data.** $^1$H-NMR data for a selected 35 compounds were in good agreement with their structure.

**3a{*1*,*1*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.34 (s, 1H, amide), 9.92 (s, 1H, amide), 8.01 (d, $J$ = 8.43 Hz, 2H, aromatic), 7.78 (d, $J$ = 8.34 Hz, 2H, aromatic), 7.65−7.62 (m, 2H, aromatic), 7.59−7.56 (m, 2H, aromatic), 7.34 (d, $J$ = 1.65 Hz, 1H, pyrrole), 7.21 (d, $J$ = 1.71 Hz, 1H, pyrrole), 7.09 (d, $J$ = 1.73 Hz, 1H, pyrrole), 6.84 (d, $J$ = 1.80 Hz, 1H, pyrrole), 3.85 (s, 3H, $CH_3$-pyrrole), 3.79 (s, 3H, $CH_3$-pyrrole), 2.05 (s, 3H, $CH_3$-acetamide). MS *m*/*z* ($ES^-$) (relative intensity) 497.91 (M − 1). HRMS $[M - H]^-$ calculated for $C_{27}H_{26}N_6O_4$ *m*/*z* 497.1937, found 497.1938.

**3a{*1*,*5*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.30 (s, 1H, amide), 9.89 (s, 1H, amide), 7.93 (d, $J$ = 8.40 Hz, 2H, aromatic), 7.72 (d, $J$ = 8.41 Hz, 2H, aromatic), 7.60 (d, $J$ = 8.22 Hz, 2H, aromatic), 7.38 (d, $J$ = 1.65 Hz, 1H, pyrrole),

7.29 (d, $J$ = 8.20 Hz, 2H, aromatic), 7.21 (d, $J$ = 1.71 Hz, 1H, pyrrole), 7.09 (d, $J$ = 1.73 Hz, 1H, pyrrole), 6.84 (d, $J$ = 1.80 Hz, 1H, pyrrole), 3.85 (s, 3H, $CH_3$-pyrrole), 3.79 (s, 3H, $CH_3$-pyrrole), 2.35 (s, 3H, $-CH_3$). MS $m/z$ ($ES^+$) (relative intensity) 456.40 (M + 1). HRMS [$M^+$] calculated for $C_{26}H_{25}N_5O_3$ $m/z$ 456.5084, found 456.5086.

**3b{*2,4*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.36 (s, 1H, amide), 9.81 (s, 1H, amide), 8.14 (bs, 1H, aromatic), 8.10 (bs, 1H, aromatic), 7.86 (d, $J$ = 7.70 Hz, 1H, aromatic), 7.82 (d, $J$ = 7.21 Hz, 1H, aromatic), 7.53 (t, $J$ = 7.73 Hz, 1H, aromatic), 7.42−7.40 (m, 1H, aromatic), 7.35 (d, $J$ = 1.41 Hz, 1H, pyrrole), 7.30−7.26 (m, 1H, aromatic), 7.20 (d, $J$ = 1.49 Hz, 1H, pyrrole), 7.09 (d, $J$ = 1.53 Hz, 1H, pyrrole), 6.96 (dd, $J$ = 8.11, 1.82 Hz, 1H, aromatic), 6.84 (d, $J$ = 1.56 Hz, 1H, pyrrole), 3.87 (s, 3H, $CH_3$-pyrrole), 3.83 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $CH_3$-pyrrole). MS $m/z$ ($ES^+$) (relative intensity) 472.50 (M + 1). HRMS [$M^+$] calculated for $C_{26}H_{25}N_5O_4$ $m/z$ 472.1907, found 472.1909.

**3b{*2,7*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.46 (s, 1H, amide), 9.91 (s, 1H, amide), 8.57 (s, 1H, aromatic), 8.27−8.23 (m, 3H, aromatic), 7.98 (t, $J$ = 7.80 Hz, 1H, aromatic), 7.81 (t, $J$ = 7.90 Hz, 1H, aromatic), 7.68−7.64 (m, 1H, aromatic), 7.57−7.56 (m, 1H, aromatic), 7.35 (d, $J$ = 1.41 Hz, 1H, pyrrole), 7.20 (d, $J$ = 1.49 Hz, 1H, pyrrole), 7.09 (d, $J$ = 1.53 Hz, 1H, pyrrole), 6.84 (d, $J$ = 1.56 Hz, 1H, pyrrole), 3.87 (s, 3H, $CH_3$-pyrrole), 3.79 (s, 3H, $CH_3$-pyrrole). MS $m/z$ ($ES^-$) (relative intensity) 485.34 (M − 1). HRMS [M − H]$^-$ calculated for $C_{25}H_{22}N_6O_5$ $m/z$ 485.1573, found 485.1584.

**3c{*3,1*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.27 (s, 1H, amide), 9.92 (s, 1H, amide), 8.02 (s, 1H, amide), 7.62−7.57 (m, 2H, aromatic), 7.43−7.41 (m, 2H, aromatic), 7.32 (d, $J$ = 3.55 Hz, 1H, furan), 7.29 (bs, 1H, pyrrole), 7.22 (bs, 1H, pyrrole), 7.07 (bs, 1H, pyrrole), 7.01 (d, $J$ = 3.55 Hz, 1H, furan), 6.84 (bs, 1H, pyrrole), 3.87 (s, 3H, $CH_3$-pyrrole), 3.81 (s, 3H, $CH_3$-pyrrole), 2.07 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 489.47 (M + 1). HRMS [$M^+$] calculated for $C_{25}H_{24}N_6O_5$ $m/z$ 489.1886, found 489.1888.

**3c{*3,2*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.20 (s, 1H, amide), 9.87 (s, 1H, amide), 8.02 (s, 1H, amide), 7.97 (bs, 1H, aromatic), 7.85 (bs, 1H, aromatic), 7.56 (t, $J$ = 8.40 Hz, 1H, aromatic), 7.39−7.35 (m, 1H, aromatic), 7.29 (bs, 1H, pyrrole), 7.22 (bs, 1H, pyrrole), 7.19 (d, $J$ = 2.91 Hz, 1H, furan), 7.07 (bs, 1H, pyrrole), 6.94 (d, $J$ = 2.92 Hz, 1H, furan), 6.84 (bs, 1H, pyrrole), 3.87 (s, 3H, $CH_3$-pyrrole), 3.81 (s, 3H, $CH_3$-pyrrole), 2.06 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 489.18 (M+1). HRMS [$M^+$] calculated for $C_{25}H_{24}N_6O_5$ $m/z$ 489.1886, found 489.1889.

**3c{*3,8*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.13 (s, 1H, amide), 9.77 (s, 1H, amide), 7.97−7.93 (m, 2H, aromatic), 7.33−7.28 (m, 2H, aromatic), 7.25 (bs, 1H, pyrrole), 7.23−7.20 (m, 1H, furan), 7.18 (bs, 1H, pyrrole), 7.13 (d, $J$ = 3.50 Hz, 1H, furan), 7.07 (bs, 1H, pyrrole), 7.04 (d, $J$ = 3.51 Hz, 1H, furan), 6.48 (bs, 1H, pyrrole), 3.86 (s, 3H, $CH_3$-pyrrole), 3.80 (s, 3H, $CH_3$-pyrrole). MS $m/z$ ($ES^+$) (relative intensity) 450.15 (M + 1). HRMS [$M^+$] calculated for $C_{23}H_{20}FN_5O_4$ $m/z$ 450.1499, found 450.1498.

**3c{*3,11*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.19 (s, 1H, amide), 9.91 (s, 1H, amide), 8.01 (bs, 1H, thiophene), 7.71−7.69 (m, 1H, thiophene), 7.61 (d, $J$ = 4.95 Hz, 1H, thiophene), 7.28 (bs, 1H, pyrrole), 7.25−7.24 (m, 1H, furan), 7.21 (bs, 1H, pyrrole), 7.07 (bs, 1H, pyrrole), 6.94−6.92 (m, 1H, furan), 6.48 (bs, 1H, pyrrole), 3.86 (s, 3H, $CH_3$-pyrrole), 3.80 (s, 3H, $CH_3$-pyrrole). MS $m/z$ ($ES^-$) (relative intensity) 436.64 (M − 1). HRMS [M − H]$^-$ calculated for $C_{21}H_{19}N_5O_4S$ $m/z$ 436.1079, found 436.1101.

**3d{*4,1*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.33 (s, 1H, amide), 9.90 (s, 1H, amide), 8.01 (s, 1H, amide), 7.60 (d, $J$ = 8.30 Hz, 2H, aromatic), 7.50 (bs, 1H, thiophene), 7.45 (bs, 1H, thiophene), 7.30 (d, $J$ = 8.31 Hz, 2H, aromatic), 7.29 (bs, 1H, pyrrole), 7.27 (bs, 1H, pyrrole), 7.10 (bs, 1H, pyrrole), 6.88 (bs, 1H, pyrrole), 3.88 (s, 3H, $CH_3$-pyrrole), 3.82 (s, 3H, $CH_3$-pyrrole), 2.04 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 505.16 (M + 1). HRMS [$M^+$] calculated for $C_{25}H_{24}N_6O_4S$ $m/z$ 505.1658, found 505.1636.

**3d{*4,2*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.37 (s, 1H, amide), 9.92 (s, 1H, amide), 8.01 (s, 1H, amide), 7.90 (d, $J$ = 3.90 Hz, 1H, thiophene), 7.72−7.66 (m, 1H, aromatic), 7.60−7.57 (m, 1H, aromatic), 7.54−7.52 (m, 1H, aromatic), 7.50 (d, $J$ = 3.87 Hz, 1H, thiophene), 7.43−7.35 (m, 1H, aromatic), 7.28 (bs, 1H, pyrrole), 7.22 (bs, 1H, pyrrole), 7.06 (bs, 1H, pyrrole), 6.85 (bs, 1H, pyrrole), 3.87 (s, 3H, $CH_3$-pyrrole), 3.81 (s, 3H, $CH_3$-pyrrole), 2.07 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 505.18 (M + 1). HRMS [$M^+$] calculated for $C_{25}H_{24}N_6O_4S$ $m/z$ 505.1658, found 505.1637.

**3d{*4,3*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.30 (s, 1H, amide), 9.91 (s, 1H, amide), 7.86 (d, $J$ = 3.76 Hz, 1H, thiophene), 7.66 (d, $J$ = 8.50 Hz, 2H, aromatic), 7.44 (d, $J$ = 3.83 Hz, 1H, thiophene), 7.26 (bs, 1H, pyrrole), 7.21 (bs, 1H, pyrrole), 7.04−7.00 (m, 2H, aromatic), 6.94 (s, 1H, pyrrole), 6.84 (s, 1H, pyrrole), 3.86 (s, 3H, $CH_3$-pyrrole), 3.83 (s, 3H, $-OCH_3$), 3.80 (s, 3H, $CH_3$-pyrrole). MS $m/z$ ($ES^+$) (relative intensity) 478.65 (M + 1). HRMS [$M^+$] calculated for $C_{24}H_{23}N_5O_4S$ $m/z$ 478.1549, found 478.1533.

**3e{*5,2*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.14 (s, 1H, amide), 9.90 (s, 1H, amide), 7.70 (bs, 1H, aromatic), 7.60 (s, 1H, thiophene), 7.55 (d, $J$ = 7.40 Hz, 1H, aromatic), 7.37 (t, $J$ = 7.81 Hz, 1H, aromatic), 7.28 (d, $J$ = 1.70 Hz, 1H, pyrrole), 7.21 (d, $J$ = 1.72 Hz, 1H, pyrrole), 7.06 (d, $J$ = 7.90 Hz, 1H, aromatic), 7.04 (d, $J$ = 1.72 Hz, 1H, pyrrole), 6.84 (d, $J$ = 1.80 Hz, 1H, pyrrole), 3.86 (s, 3H, $CH_3$-pyrrole), 3.80 (s, 3H, $CH_3$-pyrrole), 2.37 (s, 3H, $CH_3$-thiophene), 2.06 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 519.76 (M + 1). HRMS [$M^+$] calculated for $C_{26}H_{26}N_6O_4S$ $m/z$ 519.1815, found 519.1810.

**3f{*6,1*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.22 (s, 1H, amide), 9.91 (s, 1H, amide), 7.90 (d, $J$ = 8.60 Hz, 2H, aromatic), 7.73 (d, $J$ = 8.61 Hz, 2H, aromatic), 7.28 (bs, 1H, pyrrole), 7.21 (bs, 1H, pyrrole), 7.05 (bs, 1H, pyrrole), 6.85 (bs, 1H, pyrrole), 3.86 (s, 3H, $CH_3$-pyrrole), 3.80 (s, 3H, $CH_3$-pyrrole), 2.63 (s, 3H, $CH_3$-thiazole), 2.08 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^-$) (relative intensity) 518.64 (M − 1). HRMS [M − H]$^-$ calculated for $C_{25}H_{25}N_7O_4S$ $m/z$ 518.1611, found 518.1603.

**4a{*1,1*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.97 (s, 1H, amide), 7.93 (d, $J$ = 8.40 Hz, 2H, aromatic), 7.71 (d, $J$ = 8.41 Hz, 2H, aromatic), 7.68 (s, 4H, aromatic), 7.33 (s, 1H, amide), 2.06 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 255.23 (M + 1). HRMS $[M + Na]^+$ calculated for $C_{15}H_{14}N_3O_2$ $m/z$ 277.0953, found 277.0946.

**4a{*1,4*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.95 (d, $J$ = 8.40 Hz, 2H, aromatic), 7.74 (d, $J$ = 8.41 Hz, 2H, aromatic), 7.40 (t, $J$ = 7.91 Hz, 1H, aromatic), 7.28 (dd, $J$ = 7.74, 2.38 Hz, 1H, aromatic), 7.24 (bs, 1H, aromatic), 6.96 (dd, $J$ = 7.68, 3.52 Hz, 1H, aromatic), 3.83 (s, 3H, $-OCH_3$). MS $m/z$ ($ES^+$) (relative intensity) 228.05 (M + 1). HRMS $[M^+]$ calculated for $C_{14}H_{13}NO_2$ $m/z$ 228.1024, found 228.1013.

**4a{*1,5*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.01 (s, 1H, amide), 7.94 (d, $J$ = 8.40 Hz, 2H, aromatic), 7.72 (d, $J$ = 8.40 Hz, 2H, aromatic), 7.62 (d, $J$ = 8.22 Hz, 2H, aromatic), 7.34 (s, 1H, amide), 7.29 (d, $J$ = 8.21 Hz, 2H, aromatic), 2.35 (s, 3H, -$CH_3$). MS $m/z$ ($ES^+$) (relative intensity) 212.13 (M + 1). HRMS $[M^+]$ calculated for $C_{14}H_{13}NO$ $m/z$ 212.1075, found 212.1066.

**4a{*1,8*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.94 (d, $J$ = 8.40 Hz, 2H, aromatic), 7.72 (d, $J$ = 8.51 Hz, 2H, aromatic), 7.45−7.43 (m, 2H, aromatic), 7.32−7.30 (m, 2H, aromatic). MS $m/z$ ($ES^+$) (relative intensity) 216.26 (M + 1). HRMS $[M^+]$ calculated for $C_{13}H_{10}FNO$ $m/z$ 216.0825, found 216.0827.

**4a{*1,11*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 8.00−7.98 (m, 1H, thiophene), 7.92 (d, $J$ = 7.90 Hz, 2H, aromatic), 7.80 (d, $J$ = 7.91 Hz, 2H, aromatic), 7.67−7.65 (m, 1H, thiophene), 7.63−7.62 (m, 1H, thiophene). MS $m/z$ ($ES^+$) (relative intensity) 204.24 (M + 1). HRMS $[M^+]$ calculated for $C_{11}H_9NOS$ $m/z$ 204.0483, found 204.0486.

**4b{*2,1*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 10.13 (s, 1H, amide), 8.10 (s, 1H, aromatic), 8.07 (s, 1H, amide), 7.81 (d, $J$ = 7.60 Hz, 1H, aromatic), 7.78 (d, $J$ = 7.61 Hz, 1H, aromatic), 7.70−7.66 (m, 4H, aromatic), 7.52 (t, $J$ = 7.90 Hz, 1H, aromatic), 2.06 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 255.02 (M + 1). HRMS $[M^+]$ calculated for $C_{15}H_{14}N_2O_2$ $m/z$ 255.1134, found 277.1132.

**4b{*2,4*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.10 (bs, 1H, aromatic), 8.06 (bs, 1H, aromatic), 7.84 (d, $J$ = 7.70 Hz, 1H, aromatic), 7.80 (d, $J$ = 7.20 Hz, 1H, aromatic), 7.55 (t, $J$ = 7.71 Hz, 1H, aromatic), 7.44−7.40 (m, 1H, aromatic), 7.31−7.23 (m, 1H, aromatic), 6.86 (dd, $J$ = 8.10, 1.65 Hz, 1H, aromatic), 3.83 (s, 3H, -$OCH_3$). MS $m/z$ ($ES^+$) (relative intensity) 228.05 (M + 1). HRMS $[M^+]$ calculated for $C_{14}H_{13}NO_2$ $m/z$ 228.1024, found 228.1034.

**4b{*2,7*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.53 (s, 1H, aromatic), 8.24 (s, 1H, aromatic), 8.20 (d, $J$ = 7.80 Hz, 1H, aromatic), 7.93 (d, $J$ = 7.70 Hz, 2H, aromatic), 7.77 (t, $J$ = 7.92 Hz, 1H, aromatic), 7.59 (t, $J$ = 7.70 Hz, 1H, aromatic), 7.46 (s, 1H, aromatic). MS $m/z$ ($ES^+$) (relative intensity) 243.23 (M + 1). HRMS $[M^+]$ calculated for $C_{13}H_{10}N_2O_3$ $m/z$ 243.0770, found 243.0776.

**4b{*2,8*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 8.13 (bs, 1H, aromatic), 8.08 (s, 1H, amide), 7.85 (d, $J$ = 7.38 Hz, 1H, aromatic), 7.80−7.79 (m, 1H, aromatic), 7.78−7.76 (m, 2H, aromatic), 7.53 (t, $J$ = 7.72 Hz, 1H, aromatic), 7.33−7.30 (m, 2H, aromatic). MS $m/z$ ($ES^+$) (relative intensity) 216.34 (M + 1). HRMS $[M^+]$ calculated for $C_{13}H_{10}NOF$ $m/z$ 216.0825, found 216.0822.

**4b{*2,11*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 8.20 (bs, 1H, aromatic), 7.96−7.94 (m, 1H, thiophene), 7.88−7.85 (m, 1H, aromatic), 7.79 (d, $J$ = 7.67 Hz, 1H, aromatic), 7.67 (d, $J$ = 2.98 Hz, 1H, thiophene), 7.62 (d, $J$ = 3.61 Hz, 1H, thiophene), 7.49 (t, $J$ = 7.89 Hz, 1H, aromatic). MS $m/z$ ($ES^+$) (relative intensity) 204.02 (M + 1). HRMS $[M^+]$ calculated for $C_{11}H_9NOS$ $m/z$ 204.0483, found 204.0488.

**4c{*3,2*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.97 (bs, 1H, aromatic), 7.85 (bs, 1H, aromatic), 7.56 (t, $J$ = 8.40 Hz, 1H, aromatic), 7.39−7.35 (m, 1H, aromatic), 7.19 (d, $J$ = 2.91 Hz, 1H, furan), 6.94 (d, $J$ = 2.91 Hz, 1H, furan), 2.06 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 245.33 (M + 1). HRMS $[M^+]$ calculated for $C_{13}H_{12}N_2O_3$ $m/z$ 245.0926, found 245.0936.

**4c{*3,4*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.96 (s, 1H, amide), 7.47−7.46 (m, 2H, aromatic), 7.37 (t, $J$ = 8.12 Hz, 1H, aromatic), 7.15 (d, $J$ = 3.55 Hz, 1H, furan), 7.09 (d, $J$ = 3.60 Hz, 1H, furan), 6.94−6.92 (m, 1H, aromatic), 3.85 (s, 3H, -$OCH_3$). MS $m/z$ ($ES^+$) (relative intensity) 218.14 (M + 1). HRMS $[M^+]$ calculated for $C_{12}H_{11}NO_3$ $m/z$ 218.0817, found 218.0817.

**4c{*3,7*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 8.72 (bs, 1H, aromatic), 8.34 (d, $J$ = 8.39 Hz, 1H, aromatic), 8.19 (d, $J$ = 8.21 Hz, 1H, aromatic), 7.76 (t, $J$ = 8.05 Hz, 1H, aromatic), 7.37 (d, $J$ = 3.60 Hz, 1H, furan), 7.21 (d, $J$ = 3.55 Hz, 1H, furan). MS $m/z$ ($ES^+$) (relative intensity) 233.24 (M + 1). HRMS $[M^+]$ calculated for $C_{11}H_8N_2O_4$ $m/z$ 233.0564, found 233.0561.

**4c{*3,8*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.97−7.93 (m, 2H, aromatic), 7.33−7.28 (m, 2H, aromatic), 7.13 (d, $J$ = 3.50 Hz, 1H, furan), 7.04 (d, $J$ = 3.51 Hz, 1H, furan). MS $m/z$ ($ES^+$) (relative intensity) 206.12 (M + 1). HRMS $[M^+]$ calculated for $C_{11}H_8NO_2F$ $m/z$ 206.0617, found 206.0620.

**4c{*3,11*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.97 (dd, $J$ = 5.91, 1.20 Hz, 1H, thiophene), 7.68−7.66 (m, 1H, th), 7.56 (dd, $J$ = 5.49, 1.22 Hz, 1H, thiophene), 7.12 (d, $J$ = 4.05 Hz, 1H, furan), 6.86 (d, 3.40 Hz, 1H, furan). MS $m/z$ ($ES^+$) (relative intensity) 194.01 (M + 1). HRMS $[M^+]$ calculated for $C_9H_7NO_2S$ $m/z$ 194.0276, found 194.0272.

**4d{*4,1*}.** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.64 (d, $J$ = 8.30 Hz, 2H, aromatic), 7.51 (bs, 1H, thiophene), 7.42 (bs, 1H, thiophene), 7.29 (d, $J$ = 8.31 Hz, 2H, aromatic), 2.07 (s, 3H, $-CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 261.49 (M + 1). HRMS $[M^+]$ calculated for $C_{13}H_{12}N_2O_2S$ $m/z$ 261.0698, found 261.0701.

**4d{*4,2*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.95 (bs, 1H, aromatic), 7.73 (d, $J$ = 3.85 Hz, 1H, thiophene), 7.54 (d, $J$ = 7.42 Hz, 1H, aromatic), 7.43 (d, $J$ = 3.85 Hz, 1H, thiophene), 7.38−7.37 (m, 1H, aromatic), 7.35 (t, $J$ = 7.69 Hz, 1H, aromatic), 2.06 (s, 3H, $CH_3$-acetamide). MS $m/z$ ($ES^+$) (relative intensity) 261.26 (M + 1). HRMS $[M^+]$ calculated for $C_{13}H_{12}N_2O_2S$ $m/z$ 261.0698, found 261.0704.

**4d{*4,5*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.71 (d, $J$ = 3.95 Hz, 1H, thiophene), 7.58−7.56 (m, 2H, aromatic), 7.45 (d, $J$ = 3.85 Hz, 1H, thiophene), 7.24 (d, $J$ = 7.90 Hz, 2H, aromatic), 2.32 (s, 3H, $-CH_3$). MS $m/z$ ($ES^+$) (relative

intensity) 218.14 (M + 1). HRMS [$M^+$] calculated for $C_{12}H_{11}NOS$ *m/z* 218.0640, found 218.0641.

**4d{*4,7*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 8.45 (bs, 1H, aromatic), 8.20−8.18 (m, 1H, aromatic), 8.16−8.13 (m, 1H, aromatic), 7.79 (d, $J$ = 3.15 Hz, 1H, thiophene), 7.77 (d, $J$ = 3.60 Hz, 1H, thiophene), 7.74 (t, $J$ = 7.98 Hz, 1H, aromatic). MS *m/z* ($ES^+$) (relative intensity) 249.01 (M + 1). HRMS [$M^+$] calculated for $C_{11}H_8N_3O_3S$ *m/z* 249.0334, found 249.0338.

**4d{*4,8*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.73−7.72 (m, 2H, aromatic), 7.71 (d, $J$ = 3.93 Hz, 1H, thiophene), 7.47 (d, $J$ = 3.89 Hz, 1H, thiophene), 7.28−7.24 (m, 2H, aromatic). MS *m/z* ($ES^+$) (relative intensity) 222.57 (M + 1). HRMS [$M^+$] calculated for $C_{11}H_7N_3O_2SF$ *m/z* 222.0311, found 222.0311.

**4e{*5,3*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.48 (s, 1H, thiophene), 7.29 (d, $J$ = 8.75 Hz, 2H, aromatic), 7.00 (d, $J$ = 8.75 Hz, 2H, aromatic), 3.78 (s, 3H, -$OCH_3$), 2.34 (s, 3H, $CH_3$-thiophene). MS *m/z* ($ES^+$) (relative intensity) 248.11 (M+1). HRMS [$M^+$] calculated for $C_{12}H_{11}NO_2S$ *m/z* 248.0756, found 248.0754.

**4e{*5,4*}.** $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.57 (s, 1H, thiophene), 7.35 (t, $J$ = 7.90 Hz, 1H, aromatic), 6.95−6.94 (m, 1H, aromatic), 6.93−6.91 (m, 1H, aromatic), 6.90−6.88 (m, 1H, aromatic), 3.78 (s, 3H, -$OCH_3$), 2.35 (s, 3H, $CH_3$-thiophene). MS *m/z* ($ES^+$) (relative intensity) 248.26 (M + 1). HRMS [$M^+$] calculated for $C_{12}H_{11}NO_2S$ *m/z* 248.0777, found 248.0773.

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**Supporting Information Available.** $^1$H NMR spectra, LC-MS spectra, UV chromatograms of selected library **1** and **2** compounds and an image of radiofrequency-tagged Syn-Phase Lanterns placed in an Alltech column. This material is available free of charge via the Internet at http://pubs.acs.org.

## Abbreviations

AT = adenine-thymine
DCM = dichloromethane
DIC = *N*,*N*′-diisopropylcarbodiimide
DMF = dimethylformamide
HOBt = 1-hydroxybenzotriazole
HPLC = high pressure liquid chromatography
LC-MS = liquid chromatography−mass spectrometry
PIP = piperidine
RAM = rink amide linker
SAR = structure−activity relationship
TFA = trifluoroacetic acid

## References and Notes

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