Multiple Multicomponent Reactions: Unexplored Substrates, Selective Processes and Versatile Chemotypes in Biomedicine

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Abstract: Multiple multicomponent reactions rapidly assemble complex structures. Despite being very productive, the lack of selectivity and the reduced number of viable transformations restrict their general application in synthesis. Hereby, we describe a rationale for a selective version of these processes based in the preferential generation of intermediates which are less reactive than the initial substrates. In this way, applying the Groebke-Blackburn-Bienaymé reaction on a range of α-polyamino-polyazines, we prepared a family compact heterocyclic scaffolds with relevant applications in medicinal and biological chemistry (live cell imaging probes, selective binders for DNA quadruplexes and antiviral agents against human adenoviruses). The approach has general character and yields complex molecular targets in a selective, tunable and direct manner.

Introduction

Multicomponent reactions (MCRs), processes in which three or more reactants interact to yield an adduct, are fundamental in the development of synthetic methods, because of their remarkable atom and step economies, molecular variability and structural diversity.[1-3] Isocyanides are pivotal in MCRs, leading to the most fruitful processes, the Ugi and Passerini reactions being paradigmatic examples.[4,5]

Especially attractive in this context is Wessjohann's development of multiple MCRs, which further increases the performance of the original formulation. In such impressive transformations, bi(poly)functional components undergo a productive set of MCRs to assemble large adducts in a single step. Through this approach, a variety of extremely complex structures, including glycoconjugates, macrocyclic cages, peptoid-peptides, etc., was prepared.[6-9] This remarkable achievement could be upgraded if different reactions were selectively performed at the repeated functionalities. However, at the present state, all reactive functional groups (mainly linked through long alkyl chains) undergo the same transformation in an indiscriminate manner. It is worth to mention the remarkable exception of Orru's chemically distinct di-isocyanides, which was elaborated up to an 8CR.[10] Sequential versions of multiple MCRs have been conducted by either blocking an existing functional group or by gradually generating new functionalities along the process.[11,12]

The design of selective multiple MCRs (Scheme 1A) constitutes a step forward in the programmed synthesis of complex compounds. In principle, such processes should be viable, provided that the adduct from the first step would be less reactive than the initial substrate. Thus, when the first MCR takes place, the intermediate adduct would bear untouched functional groups with altered electronic properties (ie, less electrophilic) in comparison with those in the starting material, as a consequence of the initial structural modification. In this way, the reaction could proceed selectively, enabling a second MCR with a different set of inputs. Also, in cases involving non-symmetrical starting materials, the reactivity of the repeated functional groups should be kinetically distinct (Scheme 1A). Moreover, as the choice of multiple MCRs is rather limited to Ugi reactions (apart form few reported processes),[13-15] the expansion of their scope would be highly beneficial.

Furthermore, the use of heterocyclic inputs, privileged motifs in drugs, yields meaningful MCR adducts.[16] Polyamino-polyazines are attractive, yet unexplored MCR substrates. For instance, diaminopyrimidines are relevant in medicinal chemistry,[17] while melamine plays a key role in materials science.[18]

The Groebke-Blackburn-Bienaymé reaction (GBBR), which involves the acid-catalyzed interaction of α-aminoazines, aldehydes and isocyanides to yield imidazoazines (Scheme 1B),[19-21] important adducts in drug discovery,[22] represents an ideal candidate to embed in a novel highly ordered MCR process. We hereby disclose our results on selective multiple GBBRs (Scheme 1C).

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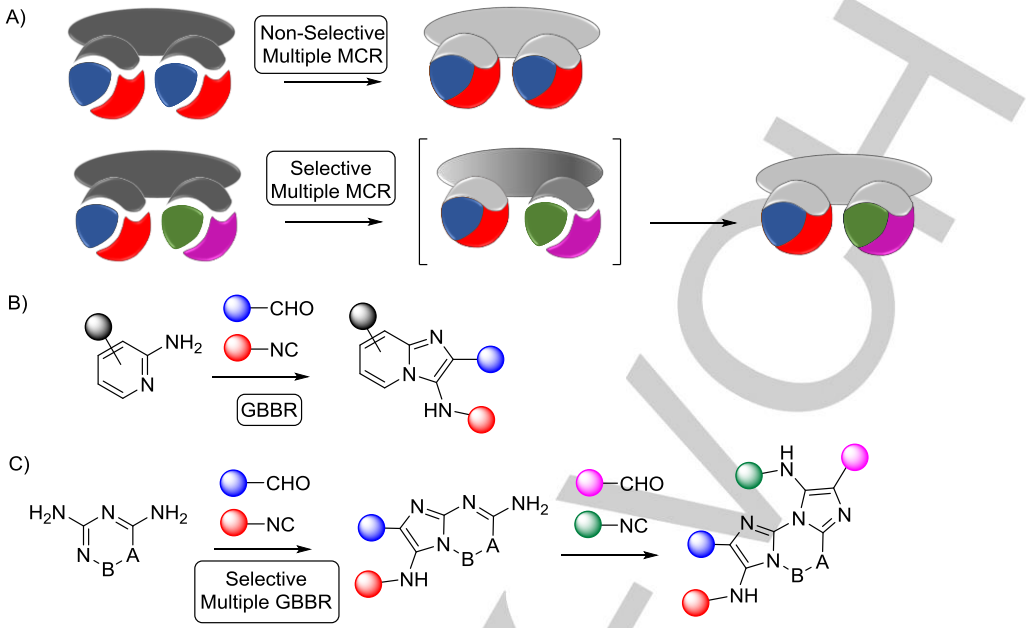


Scheme 1. Selective Multiple Multicomponent Reactions (A) Concept of selective multiple MCRs. (B) Groebke-Blackburn-Bienaymé reaction (GBBR). (C) Present work: selective multiple GBBRs.

Results and Discussion

Multiple GBBRs: Reactivity and Scope

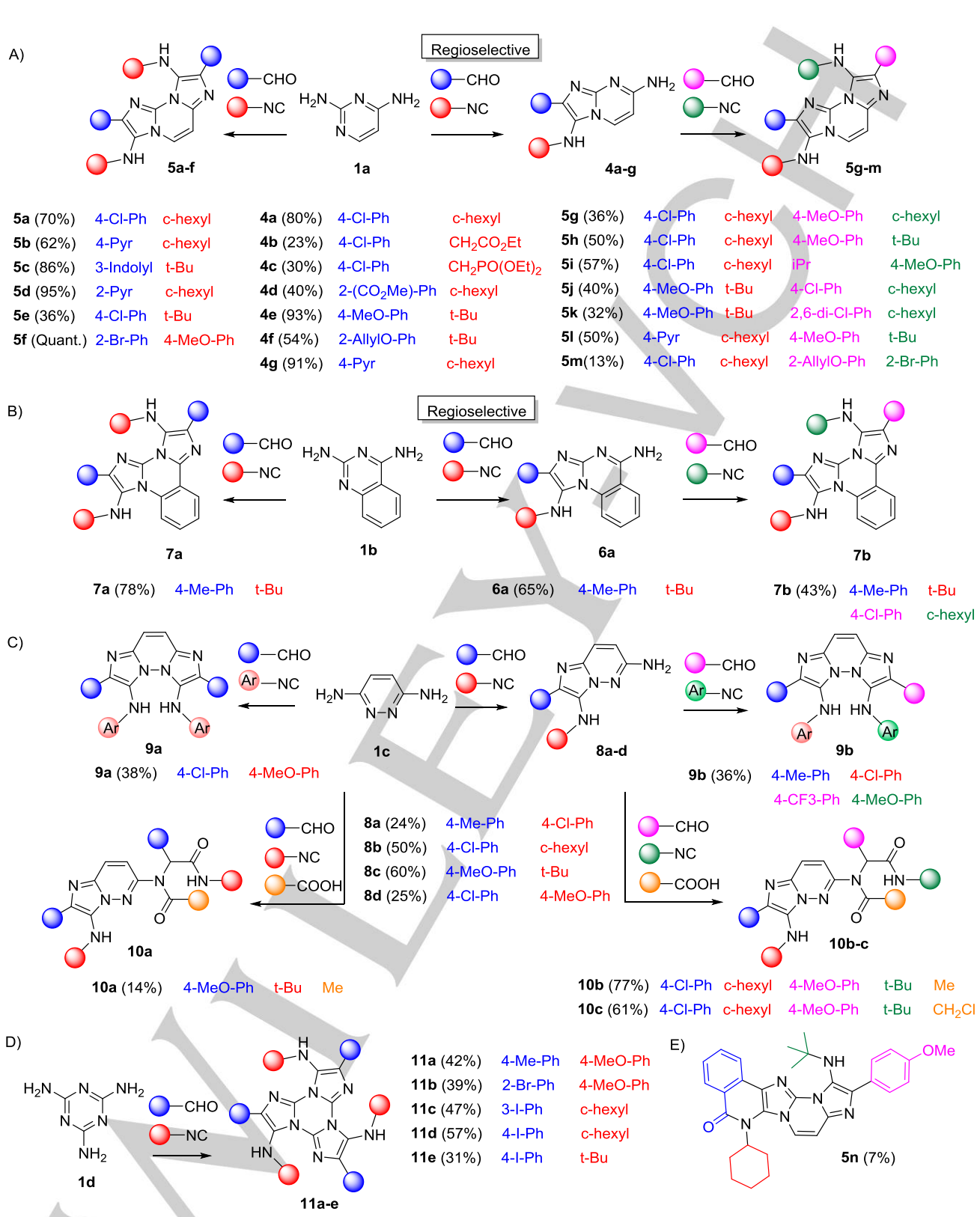
We first examined the GBBR of 4-chlorobenzaldehyde and cyclohexyl isocyanide (two equivalents each) with 2,4-diaminopyrimidine **1a**. Initial screening of reaction conditions (SI) showed that the expected multiple MCR adduct **5a** (70%) was formed under *p*-toluenesulfonic acid (PTSA) catalysis in DMF (Scheme 2A). Using a variety of common aldehydes and isocyanides, the double GBBR adducts **5a-5f** (36%-95%, Scheme 2A) were prepared. The innate selectivity of the aminoazine **1a** was then studied using mixtures of two aldehydes and two isocyanides of distinct reactivity.[23,24] Although unsymmetrical adducts were detected in over-statistical ratios, the complexity of the mixtures precluded any practical use (SI).

Thus, we addressed a sequential approach by forming one mono-GBBR adduct first, then reacting this intermediate with a distinct aldehyde-isocyanide pair to yield the double non-symmetrical compound. Equimolar amounts of aminoazine **1a**, aldehyde **2a** and isocyanide **3a** were reacted with $Yb(OTf)_3$ catalysis in Acetonitrile under microwave irradiation, leading to the selective formation of mono-GBBR adduct **4a** (80%, Scheme 2A, structure confirmed by X-Ray, SI). Then, this compound underwent a second GBBR with aldehyde **2b** and isocyanide **3a** under PTSA catalysis in DMF affording the expected product **5g** (36%, Scheme 2A, X-Ray in SI), enabling selective multiple MCRs with full control on all four diversity points of scaffold **5**. In this way, a variety of mono- and di-GBBR adducts **4a-g** (23%-93%)[25] and **5g-m** (13%-57%) respectively, were formed using this protocol (Scheme 2A). Incidentally, on the course of a GBBR upon adduct **4d**, a lactamization took place giving the pentacyclic adduct **5n** (7%, Scheme 2E).

To show the power of our approach, we prepared the 5CR adducts **5h** (50%) and **5j** (40%), displaying the same substituents in complementary positions, merely changing the order in which the MCRs were performed (Scheme 2A). The aldehyde/isocyanide scope of these multiple MCRs is basically the same found in standard GBBRs.

Next, we explored the range of the aminoazine component. Diaminoquinazoline **1b** (Scheme 2B) underwent a regioselective GBBR yielding mono-adduct **6a** (65%). In a different reaction, substrate **1b** the symmetrical bis-adduct **7a** (78%). Moreover, a second GBBR, performed upon **6a**, gave the non-symmetrical compound **7b** (43%, Scheme 2B).

Diamino-pyridazine **1c** afforded the mono GBBR adducts **8a-d** (24%-60%). The symmetrical and non-symmetrical double GBBR adducts **9a** (38%) and **9b** (36%) were also prepared (Scheme 2C). Interestingly, with substrate **1c**, the di-GBBR adducts were only formed using aromatic isocyanides, whereas bulkier ones (*tert*-butyl) failed to react, likely due to the steric clash of the proximal amines in the putative structure. Remarkably, the mono GBBR adducts **8**, upon interaction with an isocyanide, an aldehyde and a carboxylic acid underwent a standard Ugi reaction to yield the new scaffold **10.** Adduct **10a** (14%, unoptimized) was directly isolated in a one-pot process involving a spontaneous GBBR-Ugi sequence in the presence of AcOH (first catalyzing the GBBR and then reacting in the Ugi step).



Scheme 2. The scope of the multiple GBBRs A) GBBRs and double GBBRs of diaminopyrimidine **1a** and the synthesized library. B) Diaminoquinazoline **1b** in multiple GBBRs. C) GBBR, double GBBRs and GBBR-Ugi upon diaminopyridazine **1c** and the synthesized library. D) Melamine (**1d**) triple GBBR processes. E) Lactam **5n**.

Adducts **10b** (77%) and **10c** (61%), featuring five diversity points, were thus prepared from the corresponding mono adducts (Scheme 2C).

Finally, we tackled melamine (**1d**), a key key reactant with widespread use. However, its reactivity is troublesome due to its poor solubility in most organic solvents, thus having remained unexplored in MCRs. Using our PTSA method, we generated the triple adducts **11a-e** (31%-57%, Scheme 2D, **11a** X-Ray crystallography in SI). Scaffold **11** is a novel tripodal, compact, N-fused tetracyclic nucleus, conveniently synthesized in a formal 7CR, featuring the formation of nine bonds in a single step. The selective formation of melamine non-symmetrical adducts is not yet feasible and studies towards this goal are ongoing.

Post-Condensation Transformations

Post-transformations of MCR adducts can diversify the initial scaffolds, leading to complex and valuable chemotypes. In this way, our GBBR cores were modified through a number of representative reactions. The pyridine rings in adduct **5b** were alkylated with iodomethane to give salt **12** (78%, Figure 1A). This selective alkylation reflects the higher reactivity of the DMAP-like moieties. Additionally, 2,5-diketopiperazine **13** (43%), a relevant scaffold in medicinal chemistry,[26] was generated from the GBBR-Ugi adduct **10c** (Figure 1B).[27]

N-fused polycyclic structures, resembling N-doped nanographene ribbons,[28] are valuable in materials science, although their preparation entails complex multistep sequences.

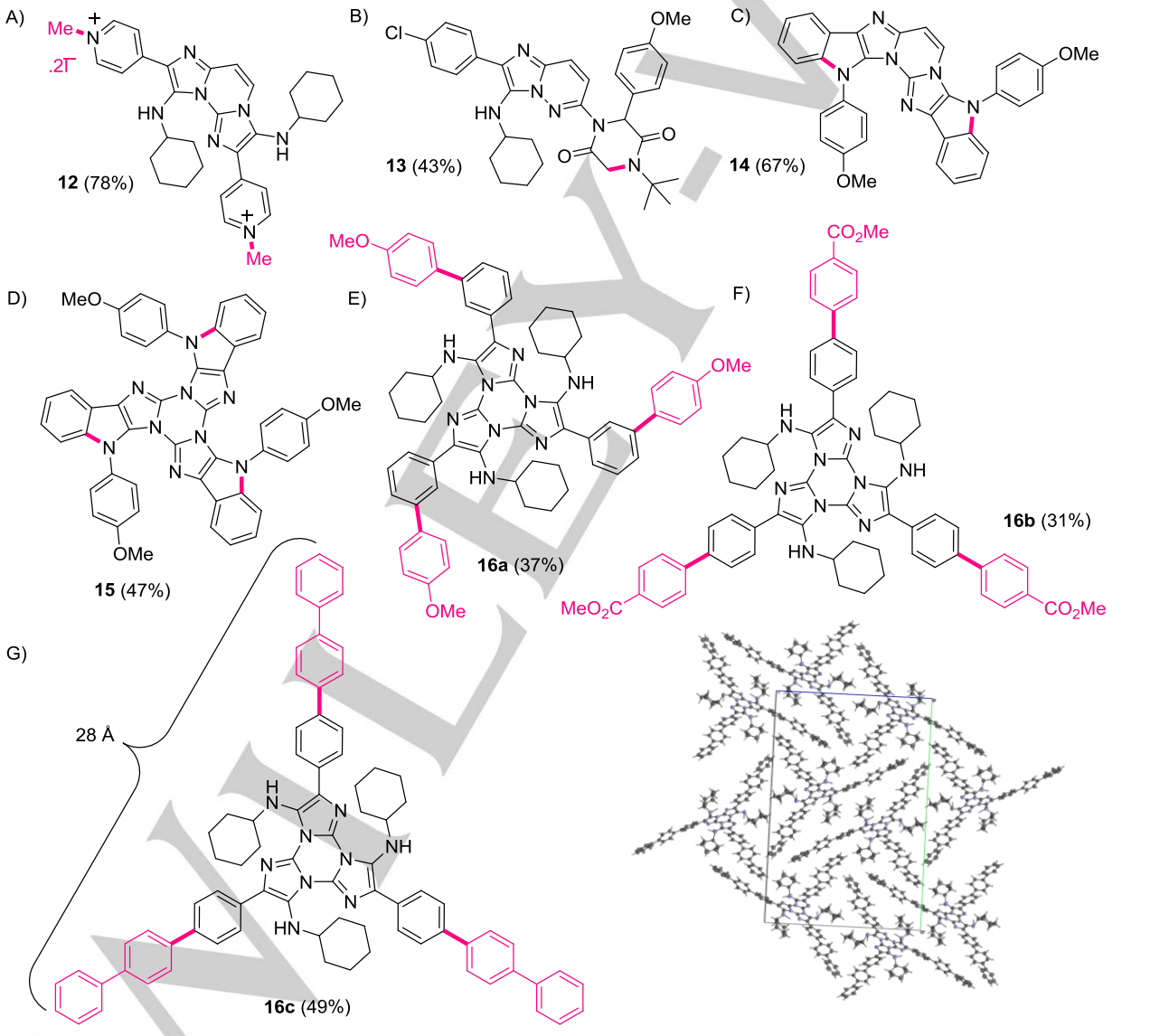


Figure 1. Post-Transformations of Multiple GBBR Adducts**.** A) Pyridinium salt **12**. B) Diketopiperazine **13**. C and D) Ullmann-type adducts **14** and **15**. E, F and G) Suzuki-Miyaura cross-coupling adducts **16a-c** and molecular packing arrangement of compound **16c**. New bonds marked in magenta, added residues in pink.

In this context, GBBR adducts were subjected to metal-catalyzed cross-couplings. Intramolecular Ullmann-type amination upon adduct **5f** yielded the heptacyclic compound **14** (67%, Figure 1C). The same transformation upon **11b** successfully gave the decacyclic radial derivative **15** (47%, Figure 1D). Tetracyclic adducts **11c-d**, fitted with aryl iodide residues, reacted with arylboronic acids via multiple Suzuki-Miyaura cross-couplings to access star-shaped compounds **16a-c** (31-49%, Figure 1E-G). Remarkably, compounds **16b-c** have nanometric dimensions, the latter reaching almost 30 Å length. Its X-Ray diffraction shows a regular molecule with a planar core and some degree of conformational freedom around the cyclohexyl residues (Figure 1G and SI). The crystal packing features an interesting docking arrangement of the central π system with intertwining of the triaryl groups (Figure S2 in SI). These examples notably demonstrate the power of the methodology to build functionalized nanosized entities through a direct bottom-up approach.

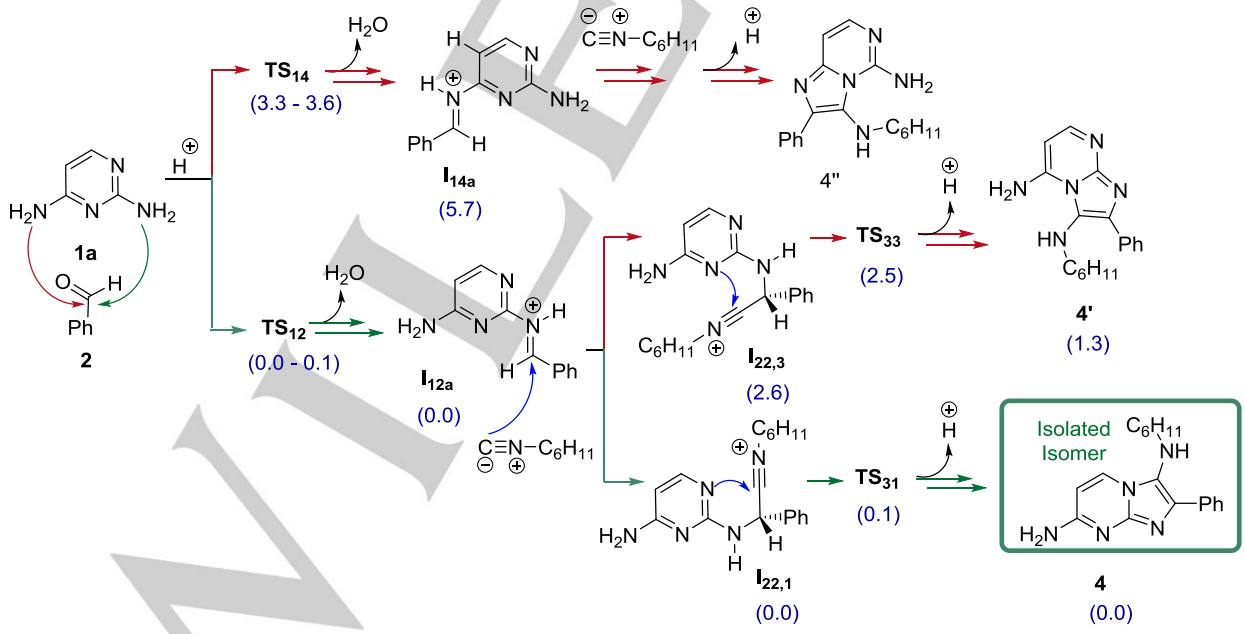
Computational Studies of Mechanism Pathways

The regioselective formation of mono-GBBR adducts 4 (Scheme 2A) is key for the synthetic usefulness of the approach. This outcome is the result of two crucial steps, the first one involving the imine formation since the preferential attack of the amino groups at positions 2 or 4 may be relevant to the selectivity (Scheme 3). To this end, M062X/6-31+G(d) calculations[29,30] (SI) were performed to identify the transition states (TSs) formed along the corresponding pathways. The TSs formed via addition of the amino group in position 2 are ≈ 3 kcal/mol more stable than the ones generated upon attack at position 4. The 2-amino tetrahedral adduct is favored by 4.6 kcal/mol relative to the corresponding amino-4 species. Finally, loss of a water molecule leads to two almost isostable protonated imine conformers, which are more stable (5.7 kcal/mol) than the 4-amino counterparts. The second step involves the cyclization of the adduct formed upon addition of the isocyanide to the imine derivative.

This process preferentially takes place through intermediate $I1_{2a}$, leading to an adduct where the nitrilium carbon atom may face either the N1 or N3 in the pyrimidine ring, the former conformer being favored by 2.6 kcal/mol. Cyclization occurs via the attack of the azine nitrogens to the nitrilium C atom through an almost barrierless process for N1, whereas for N3 the transition is less favourable. Therefore, the conformational preference of the intermediate ($I2_{2,1}$) dictates the formation of the final product **4**. Overall, the selectivity appears to arise from the combined effect of the preferential formation of one imine (from the amino group at position 2) and its subsequent isocyanide addition/cyclization via the azine N1. These results support the feasibility of selective multiple MCRs involving difunctionalized substrates.

Applications in Chemical Biology and Medicinal Chemistry

To determine the usefulness of our methodology to access and tune valuable scaffolds, we explored the performance of the synthesized compounds in a number of disciplines related to biomedicine. As a proof of concept, we intended to show that the reported approach would rend functional compounds, amenable to fast optimization.



Scheme 3. Mechanistic Rationale for the Selective Multiple GBBRs: Reaction pathways from the interaction between 2,4-diaminopyrimidine (**1a**), benzaldehyde (**2**) and cyclohexyl isocyanide. The relative free energies (kcal/mol) are shown in blue.

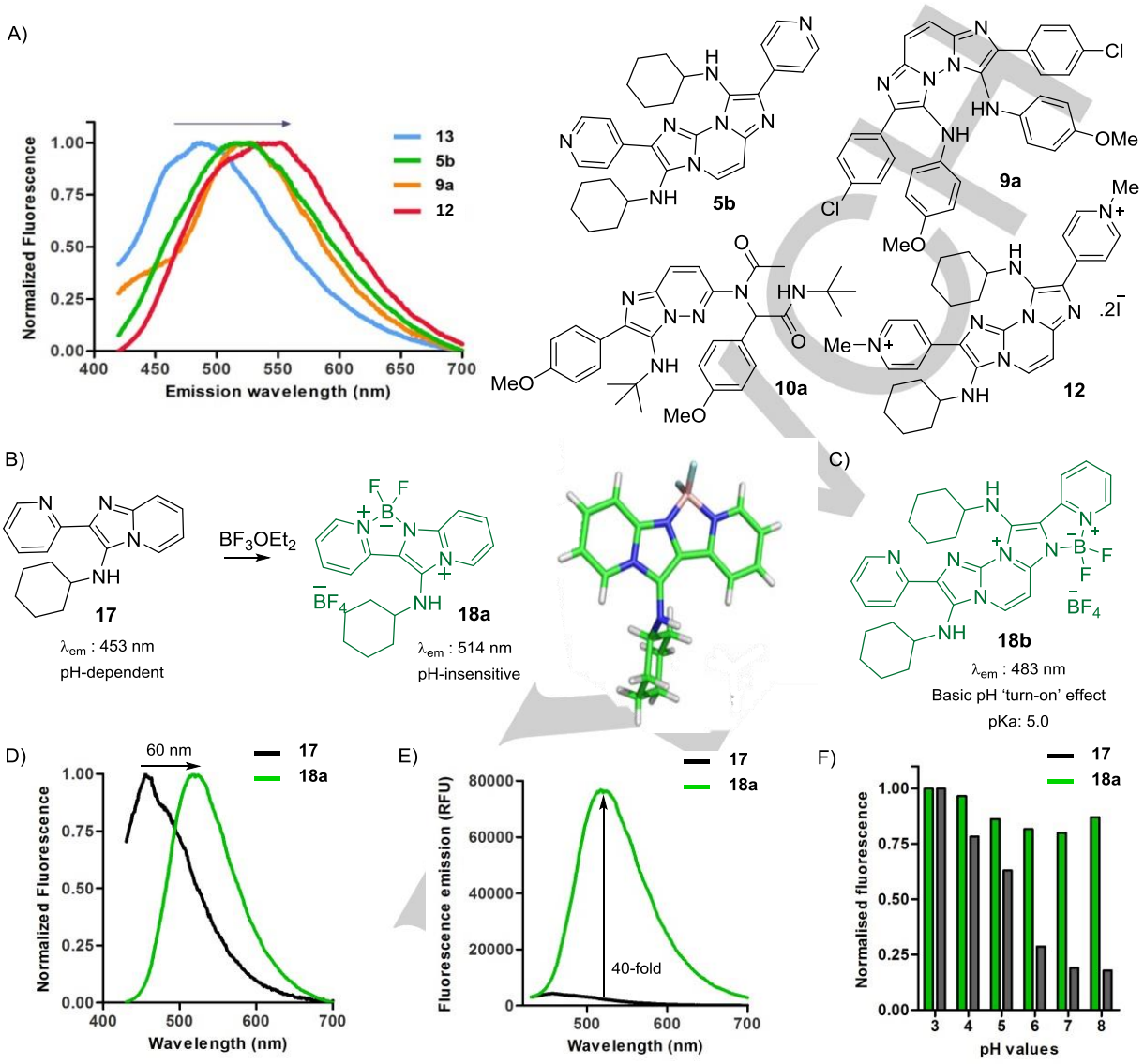


Figure 2. GBBR Adducts as Fluorophores: A) Chemical tunability of the fluorescence emission of different GBBR adducts. B) Selective formation of the 5-membered BODIPY-like fluorophore **18a** and its X-Ray diffraction. C) Probe **18b**. D, E, F) Comparative photophysical analysis of the adduct **18a** (in green) and its precursor **17** (in black).

i) Bioimaging Studies of Selected Multiple GBBR Adducts

The need for novel functional fluorophores has prompted the development of new synthetic strategies to prepare probes not accessible through classical synthesis,[31] and MCRs constitute a valuable platform to afford them.[32,33] The versatility of the novel multiple GBBRs allows the fine tuning of the structural and spectral properties of the adducts. Particularly, we controlled their red-shifted fluorescence emission wavelengths by extending their electronic conjugation with connected aryl groups (**12** vs **5b** and **9a**, Figure 2A). Our approach also enabled the introduction of electron-withdrawing and electron-donating groups at specific sites to generate push-pull fluorophores with bright fluorescence emission in the orange-to-red region (**10a**, Figure 2A). Notably, a number of GBBR adducts behaved as activatable fluorophores, with emission intensities depending on the microenvironment (Table S2, Figures S6-S7 in SI).

The excellent photophysical features of the BODIPY scaffold in bioimaging,[34,35] encouraged us to explore the generation of novel BODIPY fluorophores arising from α-pyridyl-GBBR adducts. Remarkably, model GBBR compound **17** reacted with BF_3 to selectively render complex **18a** (Figure 2B), which displays a BF_2 bridge linking the imino group and the pyridine nitrogen, suggesting that the formation of the 5-membered ring

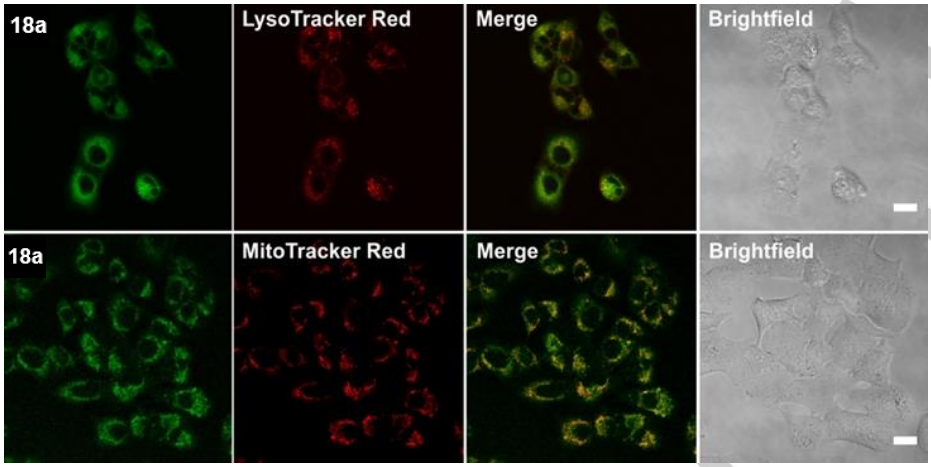


Figure 3. Brightfield and fluorescence confocal microscope images of human A549 epithelial cells upon incubation with compound **18a** (2 µM, green signal) and the commercially available trackers LysoTracker Red (red signal) and MitoTracker Red (red signal). Scale bar: 10 µm.

is faster than the conventional 6-membered BODIPY cycle.[36] Analogously, the BODIPY-like compound **18b** was obtained from the double GBBR adduct **5d** (Figure 2C).

Notably, compound **18b** showed pH-dependent fluorescence emission, with a pKa of 5.0 and brighter fluorescence in basic media, unlike the majority of BODIPY dyes. Next, the photophysical features of the probe **18a** were analyzed and compared with those of its precursor. Besides exhibiting a remarkably longer emission wavelength (453 nm to 514 nm, Figure 2D), compound **18a** featured a 40-fold higher fluorescence quantum yield, presumably due to the increased rigidification of the BODIPY core (Figure 2E). Unlike its precursor **17** (or **18b**), the fluorescence emission of compound **18a** did not show meaningful pH dependence, asserting its value as a bright green fluorophore for bioimaging assays covering a broad range of pH values (Figure 2F, SI).

We also confirmed the compatibility of probe **18a** for live-cell imaging by incubating human lung A549 epithelial cells and acquiring images under a confocal fluorescence microscope. Fluorophore **18a** showed excellent cell permeability and preferential accumulation in the mitochondria, as demonstrated by co-incubation with the commercially available LysoTracker and MitoTracker dyes (Figure 3, SI). These results indicate the suitability of multiple GBBRs to generate novel fluorescent scaffolds with excellent features for bioimaging studies.

ii) Antiviral Activity of Multiple GBBR Adducts

We determined the therapeutic potential of multiple GBBR scaffolds[22] against human adenoviruses (HAdV). While responsible for mild diseases in healthy individuals, HAdV infections are associated with high mortality in immunosuppressed patients.[37] The lack of approved specific antiviral drugs with efficacy and safety against HAdV further complicates the treatment of these patients.[38] In this context, representative GBBR adducts (Table 1 and Figure S11 in SI) were tested against HAdV using different susceptibility assays. Remarkably, five members of this chemset displayed significant anti-HAdV activity in the plaque assay, when evaluated at a concentration of 10 µM (Table 1, SI). Among these, compounds **5h** and **5n** presented a dose-dependent activity, with IC_{50} values of 1.19 and 3.42 µM, respectively, and high selectivity indexes (94 and 62, Table 1). The most cytotoxic molecule, **5b** presented the lowest selectivity index. Interestingly, compound **10b** (dose-independent, inactive below 10 µM) displayed a relevant virus yield reduction (157-fold, Table 1). In relation with frequently used antivirals, cidofovir, the drug of choice for HAdV infections, showed a significantly lower potency (IC_{50} ≈24 µM). These results are encouraging given the observed structure-activity relationships. In particular, GBBR adducts **5g** and **5h**, sharing the same diaminopyrimidine scaffold, display very different bioactivity (Table 1, SI). Also adduct **10b** is considerably more active than the close analogue **9a**. This trend strongly suggests that the bioactivity profile could be further improved through programmed exploration around this chemistry.

Table 1. Inhibitory HAdV activity of multiple GBBR adducts

Adduct	IC_{50} (µM)	CC_{50} (µM)	S.I [a]	V.Y.R [b]
5b	2,46±0,00	22,90±2,24	9	115,42±59,76
5h	1,19±0,08	112,50±2,94	94	11,54±5,98
5n	3,42±1,33	215,22±8,53	63	24,88±12,87
10a	≤ 10 [c]	62,80±12,53	---	11,54±5,98
10b	≤ 10 [c]	132,2±1,41	---	157,72±0,00

[a] Selectivity index. [b] Virus yield reduction (fold-reduction). [c] The IC50 could not be determined. **10a** and **10b** do not show a dose-dependent effect, although they are active at 10 µM.

iii) Affinity of Multiple GBBR Adducts to DNA

Since DNA displays a variety of different structural types, it is crucial to have selective binders to study their roles as a preliminary step to develop medicines.[39] Especially relevant are G-quadruplexes, because of their wide presence in the genome, mainly involved in the maintenance of chromosomes and in the transcriptional regulation of genes.[40] Topology-dependent ligands, selectively binding quadruplexes, will therefore have a significant impact on biological and medicinal chemistry.[41] Flat N-heteroaromatic motifs with polar or cationic groups are frequently found in active binders, stabilizing the G-tetrads by π-stacking and electrostatic interactions, although they are often non-selective for a defined substructure.

In this context, we explored a subset of our GBBR adducts (**5b**, **11b**, **12** and **18b**) in the interaction with model DNA oligonucleotides. Competitive dialysis experiments[42] were performed with ten oligonucleotides representing prototypical DNA structures (Table S4 in SI): single-strands, double-strands and, especially, G-quadruplexes, representing different topologies (parallel, antiparallel and hybrid), some of which are sensitive to binders causing downregulation of oncogene expression.[43] Melamine adduct **11b** interacts with all DNA sequences, being significantly more intense with the single strand T20. Compound **5b** showed a non-specific, weak affinity for several sequences (SI). However, its dimethylated salt **12** remarkably displayed a potent and selective affinity for the hybrid quadruplex 24blc (Figure 4B). The cationic nature of **12** may explain its stronger interactions in comparison with those of adduct **5b**. The charged BODIPY-adduct **18b** showed affinity again for 24bcl (Figure 4A).

To study the interaction of compound **12** with quadruplexes, we carried out fluorescence titrations of sequences GG1 and 24bcl (hybrid quadruplex) with double strand DS26 as the negative control (Figure S15 in SI). The titration curves showed that raising the relative oligonucleotide-drug concentration up to 18-fold, resulted in a significant increase (≈5-fold) in the fluorescence of the combination 24bcl-**12**. A similar, yet less potent, behaviour (≈3-fold), was detected with GG1. However, in the case of interaction with DS26, no significant change was observed (Figure 4C). Considering that oligonucleotides are non-fluorescent, and that the drug concentration is constant, the observed increase in fluorescence arises from the formation of oligomer-drug complexes.

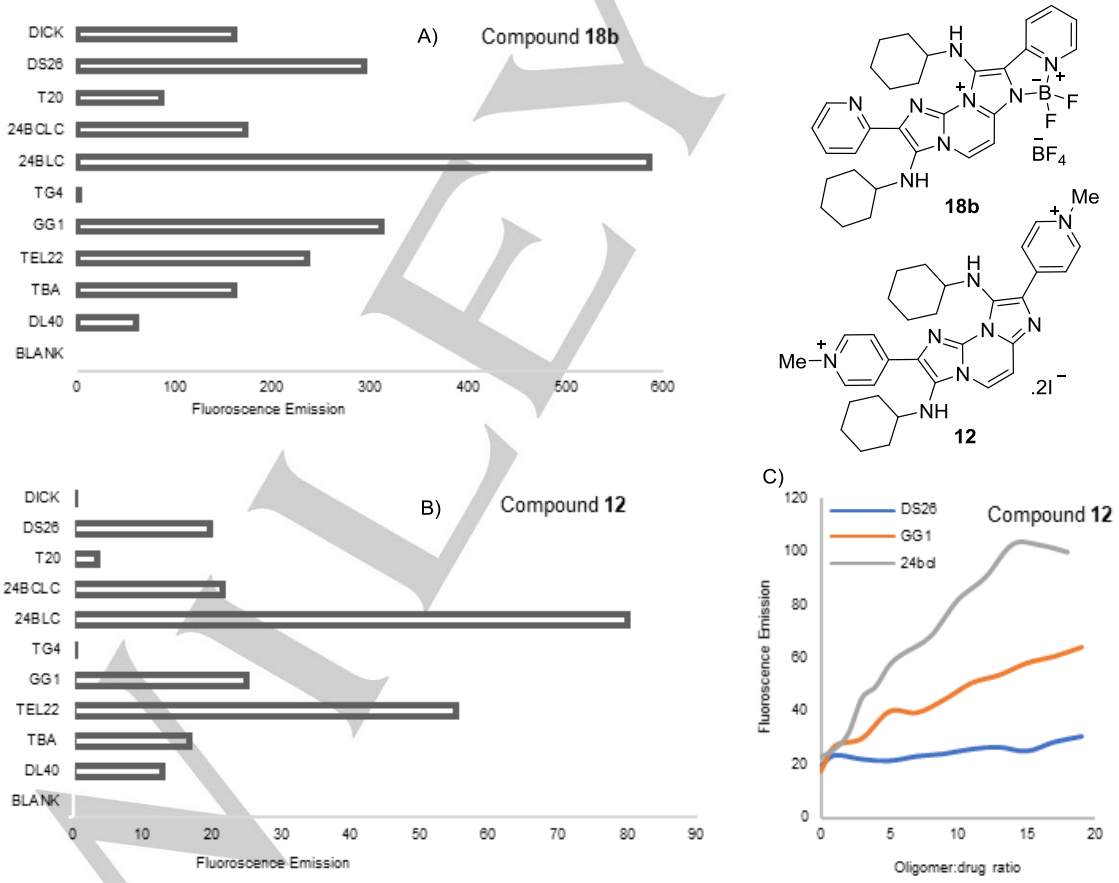


Figure 4. DNA Binding Studies of Multiple GBBR adducts: A, B) Competitive dialysis assays of ten oligonucleotide sequences with compounds **18b** and **12**. C) Fluorescence titration of a 0.2 μM solution of the GBBR adduct **12** after addition of increasing amounts of oligonucleotides DS26, GG1 and 24bcl (from 0 to 10 μM) in potassium phosphate buffer.

These results indicate that compound **12** has selective affinity to quadruplex DNA structures, especially 24bcl (Figure 4B, C) and may serve as a lead to develop new selective binders, a promising way to generate novel anticancer drugs.

Conclusions

In summary, we have developed a rationale for selective multiple MCRs, by performing GBBRs involving heterocyclic di/triamines, substantially expanding the scope for these processes. Their feasibility is based on sequential processes exploiting the higher reactivity along preferred evolutionary pathways, discriminating between nearly identical functional groups. The mechanism of these selective transformations was established by means of computational methods. This sequential mode allows the programmed incorporation of substituents at up to 5/6 diversity points. As a proof of concept, the resulting adducts, which would be impossible or extremely challenging to prepare by alternative synthetic pathways, display remarkable properties as antivirals, fluorescent probes, selective DNA binders and nanometric blocks. Moreover, their fast and controlled synthesis would enable the straightforward tuning of their properties. The general character of this approach and the applications of the ensuing scaffolds will significantly expand the reach of selective multiple MCRs in biology and medicine

Experimental Section

Full account of the experimental procedures is provided in the Supplementary Information. It includes experimental protocols, compound characterization data, copies of the spectra, X-ray description of some adducts, details on the computational studies, bioimaging experiments, antiviral studies, and DNA binding determinations.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Keywords: azines •biological activity • isocyanides• multicomponent reactions • novel scaffolds

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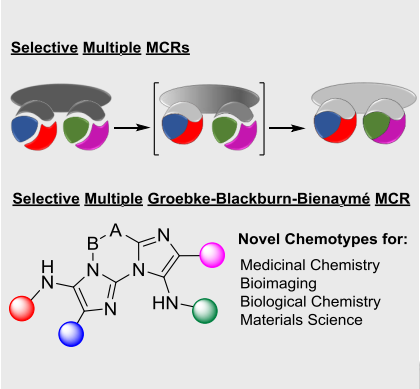
FULL PAPER

Multiple & selective: A rationale for selective multiple Multicomponent Reactions was developed and implemented in Groebke-Blackburn-Bienaymé transformations with a range of polyamino-polyazines to yield novel chemotypes, fitted with up to 5 diversity points. The generated adducts display impressive performance as live cell fluorescent probes, selective G-quadruplex binders, and antiviral agents.



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