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Parallel Supported Synthesis of Polyamine–Imidazole Conjugates

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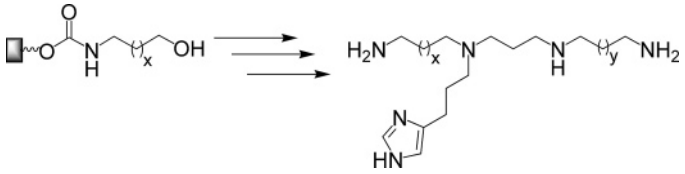
ABSTRACT



A small collection of nine polyamine–imidazole conjugates, potentially acting as RNases A mimics, has been synthesized on SynPhase lanterns using amino alcohols and diamines as building blocks. Couplings were performed via S_N2 alkylation of methanesulfonates with amines. The final introduction of *N*-4-nitrobenzyloxycarbonyldiamines allowed easy purification of the cleaved compounds.

The synthesis of molecules capable of cleaving RNA nonrandomly has found a variety of important applications in molecular biology[1,2] including as probes for investigation of nucleic acid structure in solution or in the development of new antiviral therapeutics[3] since RNA is the genetic material of many pathogenic viruses.

Several approaches have been reported ranging from antisense strategies[4] to small molecules mimicking the active center of ribonuclease A (RNase A).[2,5−8] In a previous paper,[9] we described the solution-phase synthesis of some polyamine−imidazole conjugates acting as artificial RNases A. One of these compounds exhibited a good selectivity (scission of the RNA target at a unique location). However, the strategy employed for the design of these conjugates resulted in compounds that lacked diversity since it was limited to the synthesis of symmetrical polyamine skeletons.

Thus, we decided to develop a parallel stepwise polyamine synthetic pathway on SynPhase lanterns. The two main methods of sequential synthesis of polyamine derivatives on solid supports listed in the literature are based on reduction[10,11] and alkylation[12−16] methods. The latter seemed to us to be the more suitable for generating the widest diversity, especially using a repetitive reaction of nucleophilic displacement of mesyl groups by an amine function. We previously demonstrated the efficiency of this strategy, developed on SynPhase lanterns, and which led to a small combinatorial library of triamines functionalized on an external nitrogen, starting from commercially available amino alcohols and amines.[15] In this way and on the basis of the preceding results,[9] we sought to synthesize polyamines of different lengths with an imidazole cleaving group branched

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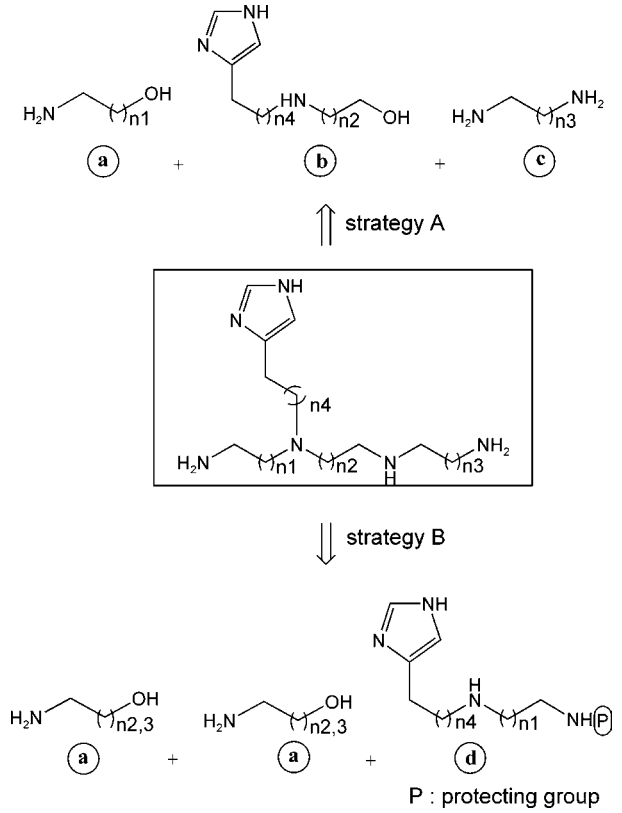
on an internal nitrogen. To obtain such compounds several strategies are possible (Scheme 1).

Scheme 1. Building Blocks and Strategies Leading to Tetramine−Imidazole Compounds Functionalized on a Central Nitrogen



In strategy A, the imidazole cleaving group is linked to the amine function of an amino alcohol (building block **b**). The other building blocks are the commercial amino alcohols **a** and the diamines **c**. For strategy B, the imidazole cleaving group is introduced on the polyamine chain through the free amine function of a monoprotected diamine **d**. The two other building blocks are the commercial amino alcohols **a**.

We selected strategy A, which specifically afforded the opportunity to introduce the functionality through building block **b** that was easily obtained by the presence of two distinct chemical functions.

Biological evaluation on RNA requires high compound purities (≥95%), and after a few preliminary attempts, we were led to suggest a process beginning with solid-supported steps (and the "split-pool" technique), followed by some solution-phase steps, to purify each sample easily. Considering the extreme hydrophilicity of polyamine derivatives and in order to facilitate purifications and to check reactions by thin-layer chromatography, we decided to link the 4-nitrobenzyloxycarbonyl protecting group[17] onto the diamine **c**. This protecting group is stable under most aqueous acid conditions and is easily removed by hydrogenolysis.[18]

We envisaged synthesizing a small pilot combinatorial library of nine individualized polyamine−imidazole conjugates starting from commercial amino alcohols (2-aminoethanol, 3-aminopropan-1-ol, 4-aminobutan-1-ol), functionalized 3-aminopropan-1-ol **2**, and *N*-4-nitrobenzyloxycarbonyl diamines **3**−**5** (1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane) (Figure 1) which were prepared according to the literature.[19,20]

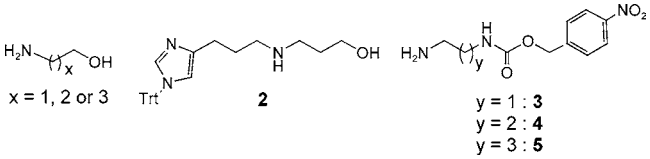
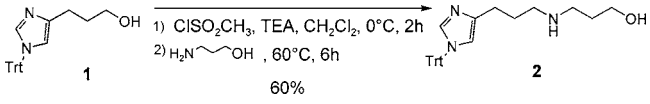


Figure 1. Building blocks employed for the construction of the small library.

Compound **2** was synthesized by the sequence shown in Scheme 2. 3-[1-(Triphenylmethyl)-1*H*-imidazol-4-yl]propan-1-ol **1** was prepared from urocanic acid.[21] Compound **1** was then converted into the corresponding methanesulfonate derivative. This latter was, without any further purification, allowed to react with an excess of 1,3-diaminopropane to afford **2** in 60% overall yield.

Scheme 2. Preparation of Building Block **2**



Once in possession of building blocks (Figure 1), the solid-supported steps of our strategy—on polystyrene Hydroxy-MethylPhenoxy (HMP) D-series SynPhase lanterns—could be undertaken (Scheme 3). To facilitate the library synthesis, a color encoding[22] directed split-pool technique was employed. Sixty HMP lanterns were divided into three groups of 20 lanterns, and each group was attached to colored stems (one color for each commercial amino alcohol, three colors in total). Each of the stem-attached lanterns was loaded with a colored cog (one color for each monoprotected diamine, three colors in total). For the first step, treatment of 60 HMP lanterns with 4-nitrophenyl chloroformate,[23] under conditions previously established in our laboratory,[24] gave 60 polymer-bound carbonates **6**.

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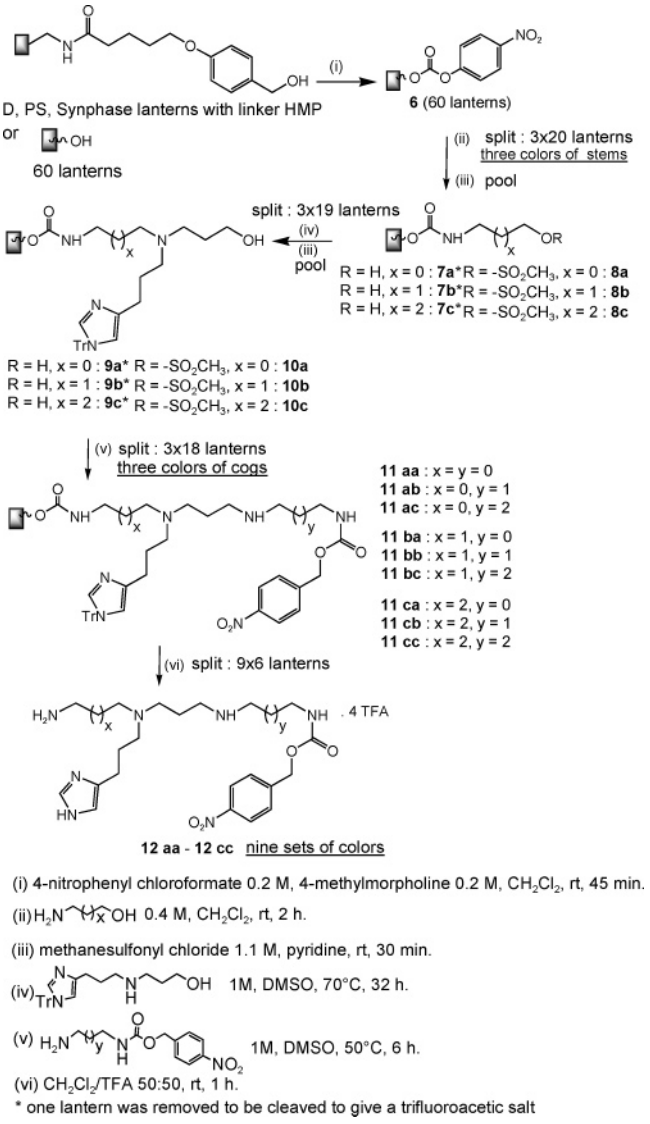
Scheme 3. Strategy A. Supported Steps

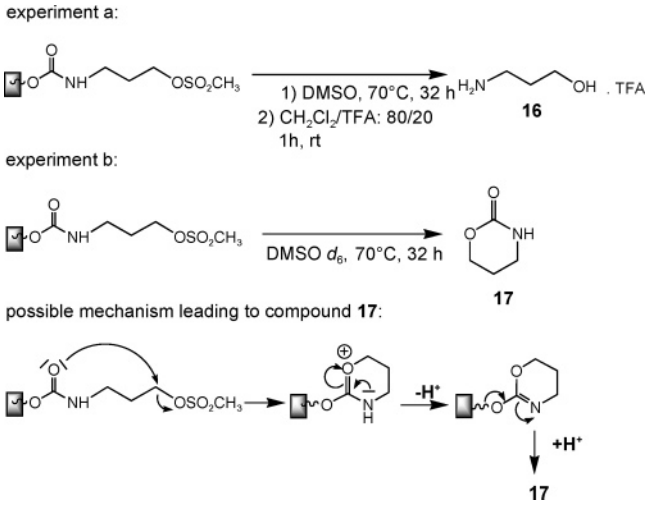


For the anchorage step, the polymer-bound carbonates **6** were converted into more stable polymer-bound carbamates: the lanterns **6** with the same color stems were joined together to react with one of the three commercial amino alcohols (three reactions in total) under conditions described in a previous paper.[24] After this reaction, all lanterns **7a**−**c** were combined for washing and drying (repeated after each step). At this level, one lantern of each batch was treated individually with a solution of trifluoroacetic acid (TFA) in CH_2Cl_2 containing 4-nitrophenol (35 μmol) as an internal standard. The ^{1}H NMR spectrum of each cleaved product was performed, and the integration ratio of the desired compound to the internal standard allowed an evaluation of the high yield (93−97%) and the high purity (>98%) of the step. The 57 supports **7a**−**c** were pooled for the mesylation step[15] to give lanterns **8a**−**c**. To facilitate manipulations and to reduce quantities of building block **2** to be introduced by nucleophilic substitution,[25] 57 lanterns **8a**−**c** were then divided into three groups of 19. The conditions previously established in our laboratory[15] (1 M concentration of nucleophilic agent at 50 °C for 6 h in anhydrous DMSO) appeared to be inappropriate for our building block 2, despite its nucleophilic secondary amine and probably because of a steric obstruction. So, we had to increase temperature (70 °C) and reaction time (32 h). Once again, one lantern of each color was removed for cleaving for ^{1}H NMR. Acceptable yields (ranging from 45 to 62%) and purities (65−69%) were obtained and are listed in the Supporting Information.

However, we noted that the shorter the mesylate analogues of lantern-bound amino alcohols, the less the substitution with building block **2** was effected (lower yield), probably because of an unwanted cleavage of the carbamate bond during the substitution step.

The lower yield was investigated with some additional experiments (Scheme 4). During experiment a, one type **8b**

Scheme 4. Formation of Compounds **16** and **17**



lantern (bearing 3-aminopropan-1-ol converted into mesylate) was submitted to conditions of substitution, but without any nucleophilic agent: after treatment with TFA (releasing from the lantern and removal of the mesyl group), compound **16** was obtained with a high purity (98%) but with a very low yield (12%), confirming a loss of compound before TFA cleavage. To analyze the product released by unexpected cleavage in the reaction medium, experiment b was undertaken: a type **8b** lantern was then introduced in the same conditions as during experiment a, but in dimethyl-d_6 sulfoxide (DMSO-d_6): compound **17** was identified by ^{1}H NMR and ^{13}C NMR, and we assume it must be formed through a rearrangement[26] described in Scheme 4. The isolation of such cyclic urethanes resulting from the thermal instability of mesylate analogues of amino alcohols (principally 2-aminoethanol and 3-aminopropan-1-ol) with amine functions converted in carbamates has already been reported in the literature.[27,28] Fifty-four lanterns **9a**−**c** were combined

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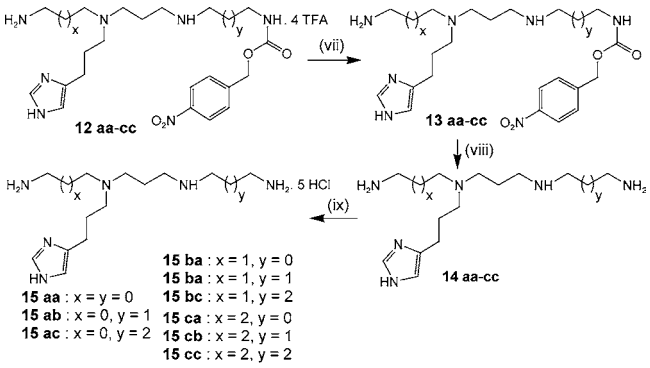
for the second step of mesylation to give lanterns **10a**−**c**. For the next combinatorial step, the 54 lanterns **10** were divided into three batches of 18 supports (each one consisted of lanterns with the same color cogs): each batch was reacted with one of the three monoprotected diamines (building blocks **3**, **4**, **5**; three batches and three reactions in total). Each lantern-bound functionalized tetramine could be simply identified by the color of the attached stems (for commercial amino alcohols) and cogs (for monoprotected diamines).

Upon combined TFA cleavage of six identical lanterns **11**, nine different tetramines **12aa**−**cc** bearing an imidazole cleaving group and protected with 4-nitrobenzyloxycarbonyl were obtained.

These tetramines could undergo a solution-phase treatment (and particularly purifications) (Scheme 5). Each trifluoroacetic salt **12aa**−**cc** was converted into its neutralized form. Because of the presence of 4-nitrobenzyloxycarbonyl group, purification by column chromatography on silica gel of each of those residues was easily accomplished using MeOH/NH_4-OH (80:20−90:10). Compounds **13aa**−**cc** were then typically obtained in 92% purity (estimated with HPLC) and 23% yield based on the initial loading of lanterns (see the Supporting Information). Deprotection of each of these nine products **13aa**−**cc** by hydrogenation over Pearlman's catalyst[18] generated the derivatives **14aa**−**cc** which reacted with hydrochloric acid to give the nine final functionalized tetramines **15aa**−**cc**. The whole library was analyzed by HPLC and HRMS and fully characterized by ^{1}H NMR and ^{13}C NMR (for selected samples an indepth structural study has been carried out using two-dimensional NMR; see the Supporting Information). These analyses confirmed the expected compounds with a good overall average yield (18%), except for compounds **15ac** and **15ba** and a high average purity (95%) (values of yields and purities are reported in the Supporting Information). An interesting point to note is that the best yields were obtained with compounds built with four carbon chains (shorter polymer-bound carbamates **8** seemed likely to undergo unexpected cleavage, and shorter *N*-4-nitrobenzyloxycarbonyl diamines could be less reactive).

Scheme 5. Strategy A. In-Solution Steps



In conclusion, an efficient protocol of parallel synthesis of polyamine derivatives was developed and involved the formation of the conjugate on D-series HMP SynPhase lanterns, via consecutive alkylations, and easy solution-phase purification of cleaved compounds thanks to a 4-nitrophenylbenzyloxycarbonyl protecting group. This versatile methodology has been used to synthesize a small library of 9 polyamine-imidazole conjugates which could be obtained in the solution phase but certainly with much lower yields and at the price of great purification effort.

The novel polyamine-imidazole conjugates are currently undergoing in vitro biological evaluation in an RNA model.

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Supporting Information Available: Experimental procedures and spectrosopic data for compounds **1**−**5**, **13aa**−**cc**, **15aa**−**cc**, **16**, and **17** and for products obtained by separated cleavages from SynPhase lanterns are available. By way of example, the in depth structural study using two-dimensional NMR (COSY, HMBC, HMQC) of compound **15bc** is reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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