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# Lithium bromide–DBU mediated synthesis of chlorophosphoramidate-activated morpholino nucleoside subunits

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ABSTRACT

An efficient and rapid protocol for the synthesis of chlorophosphoramidate-activated morpholino subunits has been developed. A unique combination of lithium bromide–DBU has been found to activate the 7′-hydroxyl toward the reaction. All the four morpholino units of adenosine, guanosine, cytosine, and thymine responded smoothly to the developed protocol.



Advancement of molecular genetics in recent years has recognized antisense compounds as potential therapeutic agents and powerful reagents for the exploration of gene functions.[1,2] Developed by Summerton, phosphorodiamidate morpholino oligonucleotides (PMOs) are one of the highly favorable third-generation antisense compounds, owing to its predictable in vivo activity in blocking mRNA translation.[3–9]

Morpholinos are synthetic DNA analogues (~25 bases long) having morpholine ring in lieu of the ribose sugar moiety and contain neutral phosphorodiamidate backbone.[6] PMOs are prepared on a solid support using an iterative two-step process, which comprises detritylation and subsequent assembling of the chlorophosphoramidate-activated subunits (Fig. 1). These activated subunits were initially synthesized by reacting the corresponding 7′-hydroxy morpholino units of adenosine (A), 5-methyl uridine (T), guanosine (G), and cytidine (C) with *N*,*N*-dimethylphosphoramidodichloride in the presence of *N*-ethylmorpholine as a base and *N*-methylimidazole as an additive.[10] The conversion was later improved by changing the base to 2,6-lutidine, and around 50% yields were reported in the case of A, C, and T.[11] However, longer reaction time (6–16 h) often poses a problem as decomposition of the product starts, and for G-morpholino, double base-protection is required to circumvent the very poor (5%) yield.[12] An alternate synthetic procedure was reported by Chen and co-workers using a strong base such as LiHMDS which gave moderate to good yields.[13] In both of the above cases, a considerable amount of the starting materials remains unreacted, and with LiHMDS, longer reaction time often leads to the formation of the phosphoric acid as the major product. Notably, synthesis of the chlorophosphoramidate intermediates for medicinally-important prodrugs also requires strong bases like *t*-BuOK[14] and LiHMDS.[15] These extremely moisture-sensitive strong bases often need hazardous preparation steps and additional precautionary measures. Because only a limited number of methods have been developed for synthesizing these base-sensitive chlorophosphoramidate-activated morpholino subunits, there is a further need for new mild and efficient synthetic procedures. In connection with our studies on modified morpholinos,[16,17] herein, we report lithium bromide-diazabicyclo-

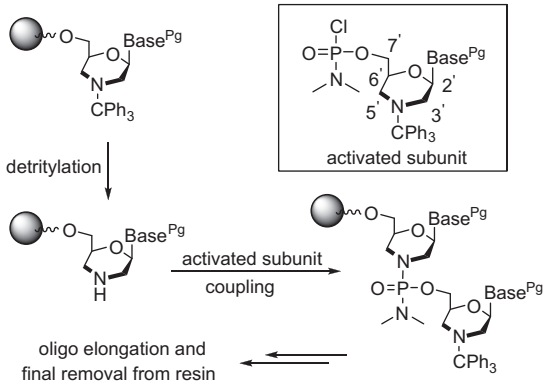


**Figure 1.** General structure and oligomerization of activated subunits.

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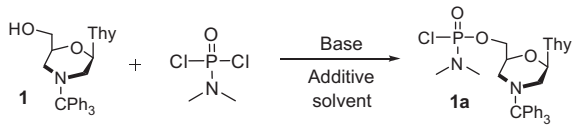
undecene (DBU) as an effective combination to synthesize the activated subunits.

The 7′-hydroxy morpholino units of A, T, G, and C were first synthesized following our established protocol.[17] To find an efficient way to carry out the transformation, we screened a number of conditions using morpholino-T unit **1** as a model substrate (Table 1).

In the absence of any additive only a trace conversion was observed when DIPEA, DBU, and DABCO (Table 1, entries 1–3) were used as bases. Use of tetrabutyl ammonium hydroxide ($n$-$Bu_4NOH$) failed to effect any conversion (Table 1, entry 4). A powerful combination of potassium carbonate-18-crown-6 was also proven to be ineffective (Table 1, entry 5). When sodium hydride was employed, at room temperature the products got decomposed (Table 1, entry 6). Careful optimization of the reaction temperature led to a pleasing 50% yield of the activated morpholino-T unit (Table 1, entry 7) but the purine morpholinos did not respond well to the NaH condition. Expectedly, another strong base $t$-BuOK was quite effective to promote the conversion (Table 1, entry 8), but was avoided because of its marked sensitivity to moisture to generate KOH which hydrolyzed the product partially to give phosphoric acid. While DIPEA and DBU were ineffective alone, in combination with $N$-methyl imidazole (30 mol %) as an additive, 19% and 26% yields of the products were obtained, respectively (Table 1, entries 9 and 10). Longer reaction time did not help the reactions to go to completion. In the presence of a catalytic amount of DMAP, DBU yielded 31% product (Table 1, entry 11) whereas $N$-methyl morpholine (NMM) failed to promote any transformation (Table 1, entry 12). Recently, the use of inorganic salts has been demonstrated to accelerate the rate of phosphorodiamidate bond formation during morpholino oligomerization.[18] However, the effect of lithium salts on the synthesis of chlorophosphoramidate-activated subunits has never been studied. The high electropositive character and small size of lithium cation make it a preferred additive in numerous organic reactions. The LiBr–DBU combination is also known to promote many organic transformations, for example, transesterification,[19] Michael addition,[20] Baylis–Hillman reaction,[21] cycloaddition reactions[22,23] etc. In our model reaction, stoichiometric amounts of LiBr in combination with 2.2 equiv of NMM, or 2,6-lutidine or DBU in acetonitrile resulted in unsatisfactory conversions (Table 1, entries 13–15). Among the non-nucleophilic bases screened, DBU has the highest p$K$a value[24] in non-aqueous solvents and seemed promising from the initial results. To our delight, when the amount of DBU and LiBr was increased to 3.2 equiv, at room temperature, the reaction was completed within 20 min and 46% yield was achieved (Table 1, entry 17). To minimize the suspected decomposition of the product, the reaction was repeated at 0 °C and better yield was obtained (Table 1, entry 18). Use of THF as solvent was also found to yield comparative result (Table 1, entry 19). The nucleoside was soluble in DCM, whereas LiBr was soluble in $CH_3CN$. Thus a 50:50 mixture of DCM and $CH_3CN$ was used as solvent and a satisfactory 61% yield for **1a** was obtained (Table 1, entry 20; best condition). To find out a better additive, other lithium halides were also screened. Interestingly, none of LiF, LiCl, and LiI exhibited satisfactory results (Table 1, entries 21–23). Lithium perchlorate ($LiClO_4$) afforded comparable yields, with a slightly lower reaction rate (Table 1, entry 24). Bromides of other representative alkali and alkaline earth metals could not promote any conversion (Table 1, entries 25–27). The unique behavior of

**Table 1**
Screening of reaction conditions[a] for the preparation of activated subunit **1a**



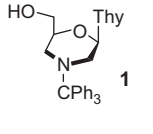
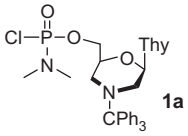
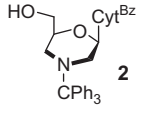
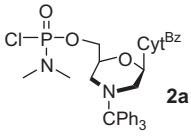
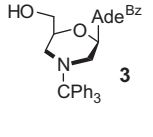
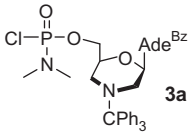
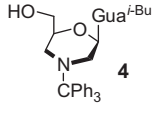
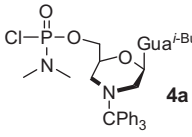
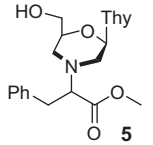
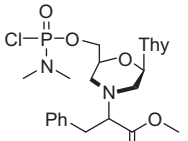
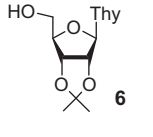
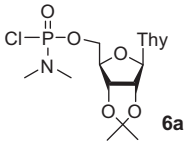

| Entry | Base (equiv) | Additive (equiv) | Solvent | Temp (°C) | Time | Yield[b] (%) |
|---|---|---|---|---|---|---|
| 1 | DIPEA (2.2) | None | DCM | rt | 12 h | Trace |
| 2 | DBU (2.2) | None | DCM | rt | 12 h | Trace |
| 3 | DABCO (2.2) | None | DCM | rt | 12 h | Trace |
| 4 | $n$-$Bu_4NOH$ (2.2) | None | THF | rt | 12 h | Trace |
| 5 | $K_2CO_3$ (2.2) | 18-Crown-6 (0.1) | THF | rt | 12 h | Trace |
| 6 | NaH (2.2)[c] | None | THF | rt/0 | 30 min | Decomp |
| 7 | NaH (2.5)[d] | None | THF | −10 | 3.5 h | 50 |
| 8 | $t$-BuOK (2.2) | None | THF | 0 | 1 h | 63 |
| 9 | DIPEA (2.2) | NMI (0.3) | DCM | rt | 3 h | 19 |
| 10 | DBU (2.5) | NMI (0.3) | DCM | rt | 4 h | 26 |
| 11 | DBU (2.5) | DMAP (0.1) | DCM | rt | 4 h | 31 |
| 12 | NMM (2.2) | DMAP (0.1) | $CH_3CN$ | rt | 12 h | Trace |
| 13 | NMM (2.2) | LiBr (1.1) | $CH_3CN$ | rt | 12 h | Trace |
| 14 | 2,6-Lutidine (2.2) | LiBr (1.1) | $CH_3CN$ | rt | 12 h | 11 |
| 15 | DBU (2.2) | LiBr (1.1) | $CH_3CN$ | rt | 12 h | 16 |
| 16 | DBU (3.2) | LiBr (2.2) | $CH_3CN$ | rt | 12 h | 21 |
| 17 | DBU (3.2) | LiBr (3.2) | $CH_3CN$ | rt | 20 min | 46 |
| 18 | DBU (3.2) | LiBr (3.2) | $CH_3CN$ | 0 | 30 min | 55 |
| 19 | DBU (3.2) | LiBr (3.2) | THF | 0 | 30 min | 47 |
| 20 | DBU (3.2)[e] | LiBr (3.2) | $CH_3CN$–DCM | 0 | 30 min | 61 |
| 21 | DBU (3.2) | LiF (3.2) | $CH_3CN$–DCM | 0 | 30 min | 6 |
| 22 | DBU (3.2) | LiCI (3.2) | $CH_3CN$–DCM | 0 | 30 min | 5 |
| 23 | DBU (3.2) | LiI (3.2) | $CH_3CN$–DCM | 0 | 30 min | 11 |
| 24 | DBU (3.2) | $LiClO_4$ (3.2) | $CH_3CN$–DCM | 0 | 30 min | 54 |
| 25 | DBU (3.2) | NaBr (3.2) | $CH_3CN$–DCM | 0 | 30 min | Trace |
| 26 | DBU (3.2) | KBr (3.2) | $CH_3CN$–DCM | 0 | 30 min | Trace |
| 27 | DBU (3.2) | $MgBr_2$ (3.2) | $CH_3CN$–DCM | 0 | 30 min | Trace |

[a] 0.05 mmol scale.
[b] Isolated yields.
[c] $NMe_2POCl_2$ added at 0 °C.
[d] $NMe_2POCl_2$ added at −30 °C.
[e] Best conditions.

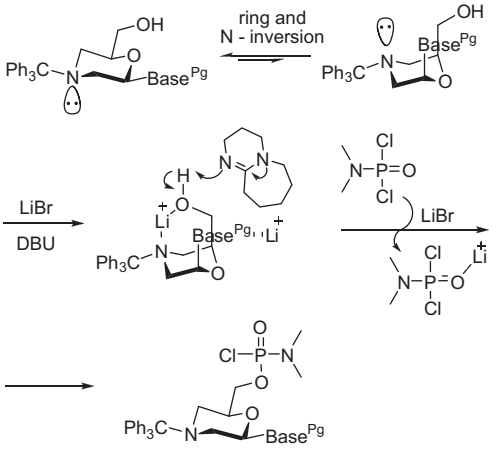
LiBr can be explained by assuming an optimum balance between lattice energy and covalent character, so that it dissociates efficiently while maintaining the coordination property of $Li^+$ in organic solvents. Effectiveness of $LiClO_4$ further proves the assumption as it also has a loosely bound $Li^+$ and low lattice energy.

Having found the optimal conditions,[25] the methodology was then extended to the other 7′-hydroxy morpholino subunits in a similar manner (Table 2). The exocyclic amine protected cytidine-morpholino subunit **2** was converted into the chlorophosphoramidate activated subunit **2a**[26] in 74% yield (Table 2, entry 2). The 7′-activated subunits of adenosine **3a** and guanosine **4a** were also obtained in high yield within a short period of time (Table 2, entries 3 and 4). It is worth mentioning here that such a clean and high yielding method has not been reported earlier. After having achieved a satisfactory way to synthesize regular activated morpholino units, we were interested to see how efficiently this methodology can be applied to other morpholino and nucleoside derivatives. A novel morpholino derivative **5**,[17b] integrating an amino acid segment in the morpholine ring responded well to furnish the corresponding product in 51% yield (entry 5). Interestingly, when we proceeded to apply the method on acetonide-protected 5-methyl uridine **6**, a large amount of starting material remained unreacted, and with 5 equiv of LiBr, only 21% yields of **6a** were obtained. The reaction also remained unsuccessful on 2′,3′-dideoxythymidine.

**Table 2**
Scope of the LiBr–DBU mediated activated subunit synthesis

| Entry | Starting material | Product | Time (min) | Yield[a] (%) |
|---|---|---|---|---|
| 1 |  **1** |  **1a** | 30 | 61 |
| 2 |  **2** |  **2a** | 25 | 74 |
| 3 |  **3** |  **3a** | 30 | 69 |
| 4 |  **4** |  **4a** | 20 | 56 |
| 5 |  **5** |  **5a** | 40 | 51 |
| 6 |  **6** |  **6a** | 60 | 21 |

[a] Isolated yields.




**Scheme 1.** Plausible mechanism for the LiBr–DBU mediated activation.

The exact mechanism of the reaction needs to be further investigated; however, a plausible mechanism (Scheme 1) can be suggested from the clues obtained. In the examples of **6** and 2′,3′-dideoxythymidine the reactions were unsatisfactory. Notably, in these, there are no ring nitrogen atoms like morpholinos which clearly indicate the role of morpholine ring-N in coordination with $Li^+$. When the ring-N was protected with electron withdrawing benzoyl group, only 9% yield of the activated unit was obtained which further proves the involvement of the ring-N. The role of $Li^+$ was unambiguously established by repeating the model reaction of **1** in the presence of lithium-coordinating solvent like HMPA, as only a marginal conversion was observed. It seems that 3.2 equiv of the lithium salt is required to get the optimum results. This can be explained as $Li^+$ also interacts with the amide functionality of acylated nucleobases and $NMe_2POCl_2$. In the presence of lithium cation, evidence for amide–imidic acid tautomerism was found in FTIR spectrum (suppression of peak at 1690 $cm^{-1}$ and emergence of sharp peak at 3566 $cm^{-1}$), when the model reaction of **1** was performed without the addition of $NMe_2POCl_2$. In another test reaction of $NMe_2POCl_2$, DBU, and LiBr, without the addition of **1**, the $^{31}P$ NMR signal shifts to downfield (from $\delta$ 19.85 to 20.00), possibly due to the coordination of $Li^+$ with $NMe_2POCl_2$. Thus mechanistically (Scheme 1), on intramolecular chelation with 7′-OH and morpholine ring-N,[27] the lithium cation forms a six-membered ring through ring and nitrogen inversion. This chelation in turn increases the acidity of the 7′-OH proton, making it easier for the base to abstract it. The alkoxide thus generated then reacts with dimethylphosphoramidodichloride to form the desired chlorophosphoramidate-activated subunit. The $Li^+$ chelation step is believed to be the rate determining step as a thermodynamically unfavorable inversion[28,29] equilibrium proceeds to the forward direction slowly.

It is noteworthy to mention here that, the chlorophosphoramidate products are not stable in solution at room temperature for longer time, especially, in the presence of any base. In all the preparation methods (including previously reported) for activated subunits, the isolated yields were often less than the conversion observed (TLC). It is therefore advisable to purify the products immediately after the reaction to obtain good yields.

In conclusion, we have developed a mild and high yielding method for the preparation of chlorophosphoramidate-activated morpholino subunits. An unprecedented application of the LiBr–DBU reagent combination has also been uncovered. Owing to the utilities of chlorophosphoramidate intermediates in the synthesis

of morpholino oligomers and medicinally important prodrugs, the present method may find potential applications.

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## Supplementary data

Supplementary data (characterization data for **1a**–**6a** and copies of spectra for the new compound **5a**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.09.126.

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25. *Typical procedure for the synthesis of chlorophosphoramidate activated morpholino subunits.* To a solution of the nucleoside (0.5 mmol) in dry DCM–$CH_3CN$ (6 ml, 50:50) was added LiBr (1.6 mmol) at 0 °C under Ar atmosphere and stirred for 2 min. Then DBU (1.6 mmol) in dry $CH_3CN$ (1 mL) was added dropwise at 0 °C to form a white precipitation. After stirring for another 2 min, dimethylphosphoramidodichloride (0.8 mmol) in $CH_3CN$ (1 mL) was added dropwise and the solution became almost clear within 15 min. The stirring was continued for the required time, the mixture was filtered through celite and the solvent was reduced under vacuum. The residue was immediately purified by silica gel flash column chromatography (230–400 mess, 7–12% acetone–DCM) to afford the activated subunit.
26. *Characterization data for compound **2a**.* White solid, mixture of diastereomers. IR (KBr): $\nu_{max}$ 1666, 1485 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.28 (t, 1H, $J$ = 11.5 Hz), 1.53 (2d, 1H, $J$ = 11.0 Hz), 2.62 (s, 3H), 2.65 (s, 3H), 3.16 (d, 1H, $J$ = 12.0 Hz), 3.59 (d, 1H, $J$ = 11.5 Hz), 4.10–4.18 (m, 2H), 4.43–4.44 (m, 1H), 6.27 (d, 1H, $J$ = 9.0 Hz), 7.18 (t, 3H, $J$ = 7.0 Hz), 7.29 (t, 6H, $J$ = 7.5 Hz), 7.36–7.53 (m, 9H), 7.57 (t, 1H, $J$ = 7.5 Hz), 7.72 (t, 1H, $J$ = 8.3 Hz), 7.88 (d, 2H, $J$ = 7.5 Hz), 8.90 (br s, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 36.7, 48.9, 52.7, 67.3, 74.8, 77.2, 82.2, 97.0, 126.7, 127.2, 127.5, 127.8, 128.0, 128.3, 128.6, 129.0, 129.3, 133.1, 133.2, 144.5, 147.1, 162.4; HRMS (ESI) $m/z$ $[M+Na]^+$, Calculated for $C_{37}H_{37}ClN_5O_5PNa$ 720.2119. Found 720.2119.
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