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Synthesis of 5-alkynylated uracil–morpholino monomers using Sonogashira coupling

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ABSTRACT

Sonogashira coupling of 5-iodo uracil–morpholino monomer with different terminal alkynes has been achieved for first time in good to excellent yields (67–86%). We have optimized the iodination conditions for the preparation of 5-iodo uracil–morpholino monomer under basic condition.

Morpholino (MO) antisense oligonucleotides (Gene Tools LLC[1]) have emerged as potent candidates for evaluating the function of a specific gene as they can inhibit the translation of the corresponding target mRNA by steric blocking and have high resistance to enzymatic degradations (Fig. 1).[2–4] MO oligomers have been routinely used for reverse-genetic studies during embryogenesis in developmental biology. The incorporation of T- or U-morpholino unit(s) into DNA or siRNA has also been reported to obtain better efficacy of these oligomers as antisense compounds.[5–7] Similarly, chimeric oligonucleotides with MO modification show better resistance toward the nuclease activity.[8] Due to the application of MO triphosphates as chain terminating reagents in DNA sequencing,[9] synthesis of these triphosphates has been reported as nucleoside analogues.[5] Apart from their antisense activity, these oligomers have also found their growing applications in nanotechnology,[10] in surface hybridization[11,12] and as neutral DNA analogues. In spite of having a wide application of morpholinos, so far there is no report on the synthesis of functionalized monomers except Gene Tools patent[1] that also describes only the synthesis of regular MOs. For academic interest as well as in continuation of our research toward morpholino synthesis, we describe herein the synthesis of 5-substituted uracil–MO monomers through C-5-alkynylation of iodo-MO monomer using Sonogashira coupling reaction.

The 5-substituted uracil derivatives could be synthesized from 5-iodo uracil–MO unit **2**, which in turn can be synthesized from uracil–MO **1**. Compound **1** was synthesized following our established protocol.[13] As there is no literature precedence toward the synthesis of iodo-substituted monomer **2**, we employed the procedures available for the iodination of uridine at C-5 and the results have been summarized in Table 1. Acid-mediated halogenation method[14] has been avoided since our MO monomers have an acid sensitive trityl group. Trityl-protection was used as it serves as a suitable protecting group during oligomer synthesis and also known to give selective *N*-tritylated product in the presence of primary OH group while synthesizing the monomer **1**.[1,15] Iodination methods reported for uridine[16a–e] have been applied on **1** (entries 1–5) and in most of the cases either starting material was recovered (entry 1) or the detritylated compound was isolated in almost quantitative yield (entries 2 and 3). In the case of NIS-mediated reaction under

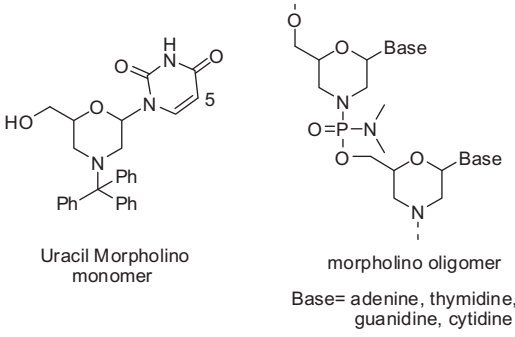

Fig. 1. Morpholino monomer and oligomer.

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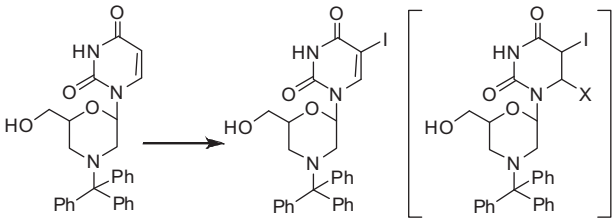
Table 1
Attempted optimization of iodination reaction



1 iodinated product; **2** addition product; X= N_3, **3a**

Entry	Iodinating agent	Solvent	Temp (°C)	Time (h)	Compound isolated
1.	I_2/K_2CO_3[a]	THF:H_2O	rt	10	**1**
2.	I_2/CAN	CH_3CN	80	8	Detritylated product
3.	NAI/CAN	CH_3CN	80	4	Detritylated product
4.	NIS	DMF	65[b]	—	**2**, 12%
5.	ICl/NaN_3	CH_3CN	0	5	**3a**, 30%

[a] 20 mol % DMAP was added as catalyst.
[b] Reaction was carried out at 200 W power for 10 min.

Table 2
Optimization of ICl mediated iodination of **1**

Entry	Iodinating agent (equiv)[a]	Base (equiv)	Temp (°C)	Product isolated[c] (%)		
				2	**3b** X=OMe	1
1.	ICl (1.2)	Et_3N (1.2)	0 to 40[b]	—	—	92
2.	ICl (1.2)	K_2CO_3 (1.2)	0 to rt	23	8	60
3.	ICl (2.0)	K_2CO_3 (1.2)	0 to rt	48	10	35
4.	ICl (2.5)	K_2CO_3 (1.25)	0 to 40	64	10	20
5.	ICl (3.0)	K_2CO_3 (1.5)	0 to 40	73	15	—

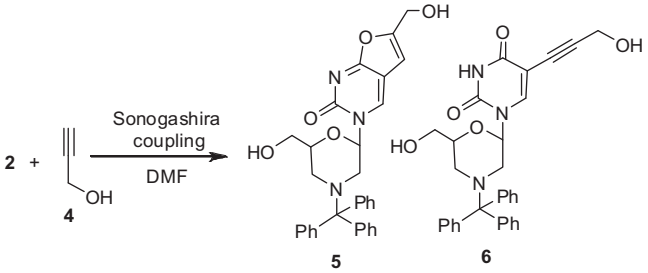
[a] Reaction was carried out in 0.5 mmol scale using MeOH as solvent.
[b] ICl was added at 0 °C then raised to 40 °C.
[c] Isolated yield.

microwave condition, the iodinated product **2** was obtained in 12% yield and the starting material **1** was also recovered (78%). When ICl/NaN_3[17] was used as iodinating agent, the addition product **3a** was isolated in 30% yield, however, the conversion was 71% based on the recovered starting material (entry 5). Interestingly, such addition product is known to form during the iodination of uridine, followed by the elimination of HN_3 to give 5-iodo-uridine. Unfortunately, in case of morpholino unit, the addition product **3a** was stable under the reaction condition even elimination did not take place in the presence of Et_3N. When the reaction was carried out at room temperature, formation of detritylated product was predominant.

Another procedure was reported[18] for iodination of 2′,3′-dideoxy uridine involving the treatment with ICl/MeOH followed by NH_4OH to give the corresponding iodo-derivative. Following their condition detritylation was noticed in our case at rt. Then we thought to introduce a base along with ICl/MeOH in the same reaction pot and the results have been summarized in Table 2. Accordingly, we tried with the combination of ICl and Et_3N (entry 1) but the starting material was recovered probably because Et_3N was forming a complex with ICl due to its Lewis base character. Replacement of Et_3N by K_2CO_3 gave the iodinated product **2** along with the addition product **3b** (entry 2). Increasing the equivalent of ICl gave a better yield of the iodinated product **2** whereas the yield of the addition product **3b** was same (entries 3 and 4). We optimized that the presence of 1.5 equiv of K_2CO_3 and 3.0 equiv of ICl in MeOH gave the best result where the starting material was consumed completely (entry 5). Though the addition product **3b** was isolated in 15% yield, **3b** was readily converted into the desired product **2**[19] in the presence of Et_3N in CH_2Cl_2 at rt. The combined yield was found to be 83%. During the iodination we realized that for every equivalent of ICl exactly half its equivalent of K_2CO_3 was needed to obtain the iodination product and to stop detritylation. Surprisingly, the combination of ICl/MeOH in the presence of K_2CO_3 did not react with 2′,3′-dideoxy uridine[18] whereas, it gave the best result in case of morpholino unit.

We next optimized the Sonogashira reaction of **2** with propargyl alcohol **4** (Table 3). Our initial attempt of the Sonogashira reaction[20] of **2** with **4** gave exclusively the cyclized product **5** in the presence of $Pd(PPh_3)_2Cl_2$ (entry 1). When Pd(0) was employed as

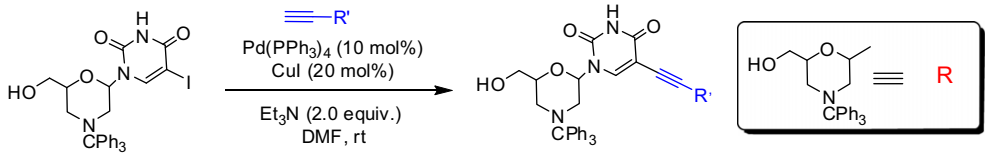
Table 3
Optimization of Sonogashira condition

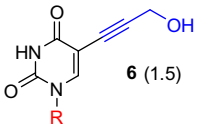
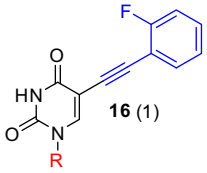
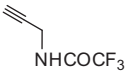
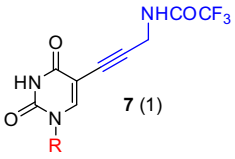
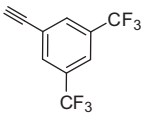
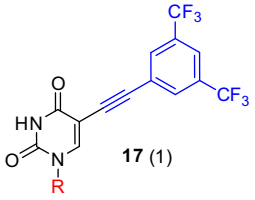
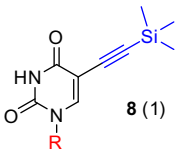
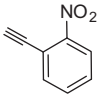
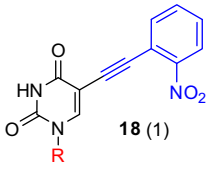
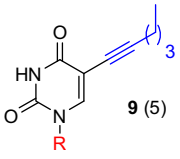
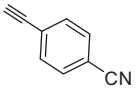
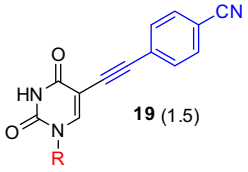
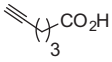
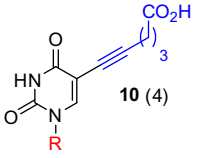
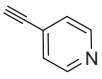
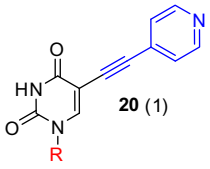
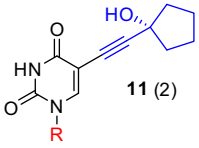
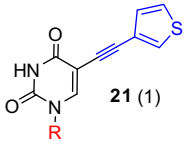
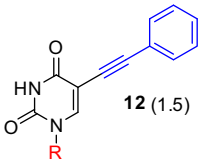
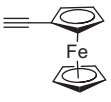
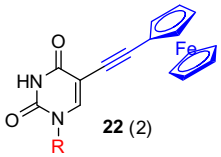
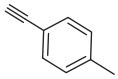
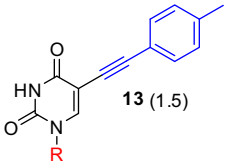
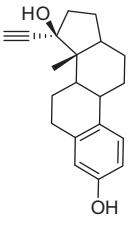
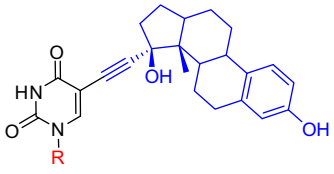


5 **6**

Entry	Palladium catalyst (mol %)	Copper source (mol %)	Product	Yield (%)
1.	$Pd(PPh_3)_2Cl_2$ (5)	CuI	**5**	63
2.	$Pd(PPh_3)_4$ (5)	CuI	**6**	38
3.	$Pd(PPh_3)_4$ (10)	CuI	**6**	86

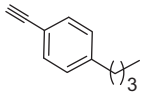
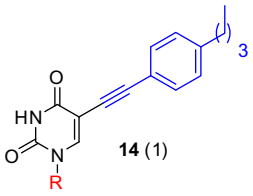
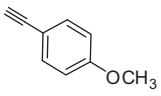
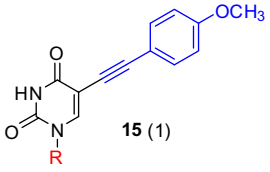
Table 4
Sonogashira coupling of 5-iodo uracil–morpholino **2** with different terminal alkynes



Entry	Alkyne	Product, time (h)	Yield	Entry	Alkyne	Product, time (h)	Yield
1		 **6** (1.5)	86	11		 **16** (1)	79
2		 **7** (1)	86	12		 **17** (1)	74
3		 **8** (1)	82	13		 **18** (1)	78
4		 **9** (5)	76	14		 **19** (1.5)	68
5		 **10** (4)	67	15		 **20** (1)	68
6		 **11** (2)	68	16		 **21** (1)	82
7		 **12** (1.5)	78	17		 **22** (2)	80
8		 **13** (1.5)	77	18		 **23** (3)	83

(*continued on next page*)

Table 4 (*continued*)

Entry	Alkyne	Product, time (h)	Yield	Entry	Alkyne	Product, time (h)	Yield
9		 **14** (1)	77				
10		 **15** (1)	77				

a catalyst, we isolated the desired product **6** though in poor yield (38%). Finally, 10 mol % of the catalyst, 20 mol % of CuI, and 2 equiv of Et_3N gave **6**[21] in excellent yield.

The optimized condition was then applied to the coupling of **2** with a wide range of electronically and structurally different alkynes (Table 4). Functionalized alkynes have been incorporated at the 5-position of **2** as the products could be used for further derivatization. Particularly propargylamine substituted monomer **7** (entry 2) is known to undergo guanidylation to give cationic oligomers.[13] Trimethylsilylacetylene-substituted monomer **19** could be further coupled at terminal alkyne after TMS-deprotection (entry 3). Arylalkynes containing both electron donating and electron withdrawing groups underwent coupling smoothly to give the corresponding 5-substituted U–morpholino monomers in very good yields (entries 7–14). In order to have a wide range of substrates, we coupled **2** with heterocyclic acetylenes and obtained the products (**20** and **21**) in excellent yields (entries 15 and 16). Likewise, ethynylferrocene and sterically hindered acetylene reacted in an efficient manner with **2** to give the products **22** and **23** in excellent yields (entry 17 and 18).

In summary, we are the first to report the synthesis of various types of alkynyl-substituted uracil–morpholino monomers. Such functionalized monomers could be useful for the synthesis of functionalized MO oligomers and also could be the potential nucleotide analogues for biomedical applications. In this direction, we also standardized the iodination method to obtain the 5-iodo U–MO monomer. Work toward the synthesis of functionalized oligomer is now underway and results will be reported in due course.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.05.141.

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19. *Preparation and characterization of compound* ***2***: To a stirred solution of **1** (1.2 g, 2.5 mmol) in dry MeOH (10.0 mL) was added K_2CO_3 (0.53 g, 3.8 mmol) under argon atmosphere. The reaction mixture was cooled to 0 °C, followed by dropwise addition of ICl (7.5 mL, 1.0 M solution in CH_2Cl_2). The reaction mixture was warmed to 40 °C and heated for a period of 1 h in dark. The reaction was monitored by TLC. Reaction mixture was evaporated to dryness, extracted with CH_2Cl_2 (2 × 30 mL). The organic layers were washed with H_2O (2 × 15 mL), and the excess iodine was removed by washing with half saturated sodium thiosulfate (20 mL). The organic layer was further washed with water (20 mL), half saturated brine (20 mL) and dried over Na_2SO_4. The organic extract was concentrated in vacuo to obtain a colorless solid. The crude mass obtained was dissolved in CH_2Cl_2 (20 mL) followed by the addition of Et_3N (2.0 mL) and left for overnight stirring. Reaction mixture was concentrated in vacuo and purified by column chromatography (100–200 mesh silica gel) using MeOH in CH_2Cl_2 as eluent to get compound **2** (1.26 g, 83%) as colorless solid. R_f (19:1, CH_2Cl_2/MeOH) = 0.51. IR (neat): ν 3385, 1687, 1681, 1448, 1265, 750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.38–1.42 (1H, t, *J* = 9.5 Hz), 1.44–1.49 (1H, t, *J* = 11.0 Hz), 2.00 (1H, br s), 3.09–3.11 (1H, dd, *J* = 12.0, 4.0 Hz), 3.38–3.41 (1H, d, *J* = 11.5 Hz), 3.58–3.64 (2H, m), 4.27–4.31 (1H, m), 6.11–6.13 (1H, dd, *J* = 9.5, 2.0 Hz), 7.18–7.21 (3H, m), 7.29–7.32 (6H, m), 7.46 (5H, br s), 7.59 (1H, s), 8.71 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 48.92, 52.46, 63.74, 68.27, 78.21, 81.29, 92.37, 126.80, 128.12, 129.19, 144.38, 149.31, 159.66; HRMS (ESI) $(M+Na)^+$ Calcd for $C_{28}H_{26}IN_3O_4Na^+$ = 618.0866. Found 618.0868.
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21. *Preparation and characterization of compound* ***6***: Compound **2** (0.184 g, 0.31 mmol) was dissolved in dry DMF (4.0 mL) followed by the addition of propargyl alcohol (0.052 g, 0.92 mmol) and Et_3N (85 μL, 0.62 mmol). The reaction mixture was degassed with argon for 15 min. To the reaction mixture were added $Pd(PPh_3)_4$ (0.036 g, 0.031 mmol) and CuI (0.012 g, 0.062 mmol) and stirred under argon atmosphere for a period of 1.5 h. The reaction mixture was diluted with EtOAc (10 mL), washed with water (3 × 10 mL), brine (5 mL). The reaction mixture was dried over Na_2SO_4, concentrated in vacuo and purified by column chromatography (100–200 mesh silica gel) using MeOH in CH_2Cl_2 as eluent to get compound **17** (0.146 g, 86%) as brown solid. R_f (1:1,

petroleum ether/EtOAc) = 0.38, IR (neat/$CHCl_3$): ν = 3078, 3054, 2217, 1702, 1698, 1510, 1247, 750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 1.34–1.38 (1H, t, J = 10.5 Hz), 1.41–1.46 (1H, t, J = 11.0 Hz), 2.11 (1H, br s), 3.03–3.05 (1H, d, J = 11.5 Hz), 3.35–3.37 (1H, d, J = 11.0 Hz), 3.47–3.62 (2H, m), 4.24–4.28 (3H, m), 6.08–6.10 (1H, dd, J = 8.0, 1.0 Hz), 7.16–7.18 (3H, m), 7.26–7.29 (6H, m), 7.45–7.52 (6H, m), 9.53 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 48.69, 51.19, 52.36, 63.55, 76.05, 77.94, 81.23, 92.87, 99.32, 126.73, 128.10, 129.30, 143.12, 148.89, 162.02. HRMS (ESI) $(M+Na)^+$ Calcd for $C_{31}H_{29}N_3O_5Na^+$ = 546.2005. Found 546.2003.