



5-(Benzylmercapto)-1*H*-tetrazole as activator for 2'-*O*-TBDMS phosphoramidite building blocks in RNA synthesis

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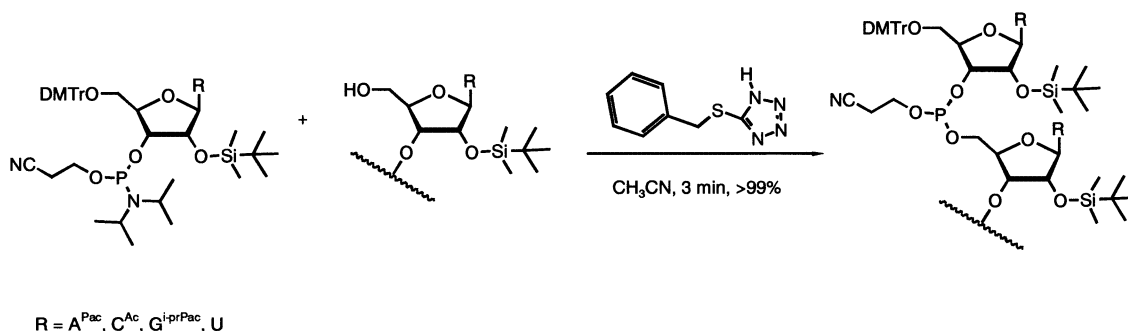
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Abstract—An improved method for the preparation of 5-(benzylmercapto)-1*H*-tetrazole as activator in RNA synthesis is described. Reaction of benzylthiocyanate with azide delivers the corresponding tetrazole with 72% yield under optimised conditions. We demonstrate that 5-(benzylmercapto)-1*H*-tetrazole is a superior activator of 2'-*O*-TBDMS phosphoramidite building blocks. Compared to routinely used 1*H*-tetrazole, application of a 0.25 M 5-(benzylmercapto)-1*H*-tetrazole solution in acetonitrile allows for higher coupling yields (>99%), lower coupling times (3 min) and reduced excess of phosphoramidites in solution over the solid-phase nucleotides (8-fold). © 2002 Elsevier Science Ltd. All rights reserved.

The chemical synthesis of RNA oligomers is less facile than DNA synthesis. In contrast to DNA monomer building blocks, ribose phosphoramidites have a 2'-hydroxyl group, which requires additional protection and which may sterically interfere with the coupling reaction. Among the 2'-*O*-protecting groups which have been suggested,¹ the (*tert*-butyl)-dimethylsilyl (TBDMS) group has found widest application. The coupling of 2'-*O*-TBDMS protected monomers under standard tetrazole activation takes 12 to 15 min with yields in the range of 97%. Strategies for improvement of RNA coupling yields involve either new 2'-*O*-protecting groups which are sterically less demanding,² or

activation reagents, which are more powerful than the routinely used 1*H*-tetrazole.^{3,4}

We have explored the potential of 5-(benzylmercapto)-1*H*-tetrazole as activating reagent in RNA synthesis (Scheme 1). Even though, this tetrazole derivative was mentioned before in connection with 2'-*O*-[(triisopropylsilyl)oxy]methyl (TOM) monomers,² to our knowledge it has never been evaluated with the commercially widely available and more routinely used TBDMS building blocks. Using 400 µl of a 0.25 M solution of this tetrazole derivative in acetonitrile (corresponding to a 100-fold excess) and a 8-fold excess of dissolved



Scheme 1. RNA phosphoramidite coupling reaction with 5-(benzylmercapto)-1*H*-tetrazole activation. Pac: phenoxyacetyl, Ac: acetyl, *i*-Pr-Pac: 3-isopropyl-phenoxyacetyl

Keywords: RNA synthesis; activator; 5-(benzylmercapto)-1*H*-tetrazole.

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phosphoramidites over the solid-phase nucleotide, we obtained average coupling yields >99%. HPLC traces of the crude synthesis products clearly show a main peak for each oligoribonucleotide trityl-off (Fig. 1, RNA 1, 2 and 3 represent a 25, 29 and 42 mer, respectively). These results demonstrate that the postulated problem in TBDMS chemistry due to sterical interfering of the TBDMS group with the coupling reaction obviously can be overcome by the more potent activator. It is not clear from the literature,² what coupling yields have been obtained applying TOM chemistry with usual tetrazole activation. Possibly the enhancement of cou-

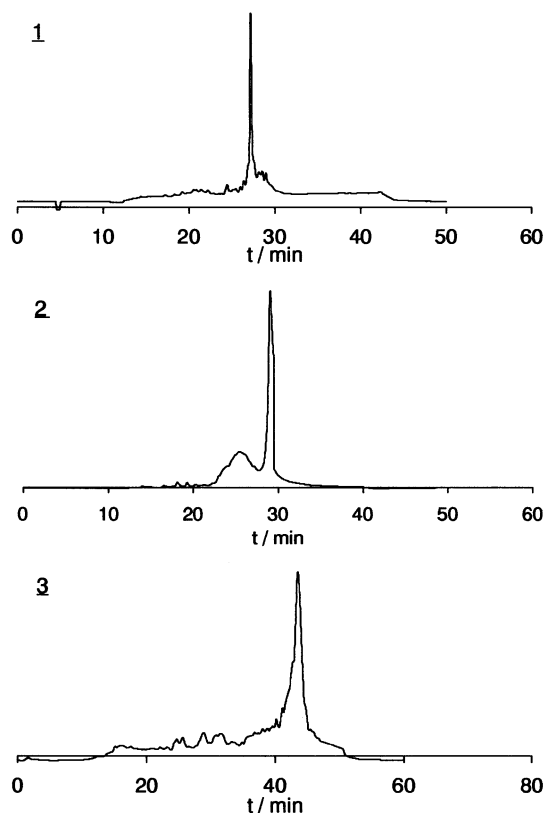


Figure 1. HPLC traces of crude RNA **1** (CAG AAA UCA CCG AAA GGU GAA CCA G), **2** (AUC UCU CUU UCU CCC UGA CAG UCC AGA AA) and **3** (ACC AGA GAA ACA CAG CUA AGG AUG AAA GUC UAU GCU GGU AUA UUA CCU GGU). Synthesis of oligomers was carried out on 1- μ mol scale with a Pharmacia GeneAssembler as reported previously⁶ with the following changes: activation reagent: 0.25 M solution of (5-benzylmercapto)-1*H*-tetrazole in acetonitrile, coupling time: 3 min, 8-fold excess of phosphoramidites. Average coupling yields were 99.5% for **1**, 99.2% for **2**, 99.1% for **3**. HPLC-conditions: Ion exchange HPLC on PL-SAX 1000 (Polymer Laboratories) with buffer A: 20 mM sodium acetate (pH 6.5)/20% CH₃CN and buffer B: 20 mM sodium acetate (pH 6.5)/20% CH₃CN, 1 M NaCl, flow rate: 1 ml/min at 0%B for 2 min, followed by a linear gradient of 0–100% B over 40 min (**1** and **2**), or on MonoQ (Pharmacia) with buffer A: 10 mM Na₃PO₄ (pH 11.5) and buffer B: 10 mM Na₃PO₄ (pH 11.5), 1M NaCl, flow rate: 1 ml/min at 0%B over 5 min and a linear gradient of 0–100%B over 50 min (**3**).

pling yields generally is dependent on 5-(benzylmercapto)-1*H*-tetrazole rather than on the nature of the 2'-*O*-protecting group. Thus, in our opinion both protecting groups can be used with a small advantage on the TBDMS side, 2'-*O*-TBDMS protected phosphoramidite building blocks are somewhat cheaper and more widely available.

Comparison of 5-(benzylmercapto)-1*H*-tetrazole to other known activators revealed the following order in coupling yields of 2'-*O*-TBDMS phosphoramidites: 5-(benzylmercapto)-1*H*-tetrazole > 5-(ethylmercapto)-1*H*-tetrazole > 4,5-dicyanoimidazole > 1*H*-tetrazole. The high activation potential of 5-(benzylmercapto)-1*H*-tetrazole is very likely based on its higher acidity (1*H*-tetrazole: p*K*_a = 4.89; 5-(ethylmercapto)-1*H*-tetrazole: p*K*_a = 4.28; 5-(benzylmercapto)-1*H*-tetrazole: p*K*_a = 4.08).⁵ Thus, in the first step of activation the trivalent phosphorus is more readily protonated and subsequently the coupling reaction is accelerated. Due to the aromatic substituent, hydrophilicity of 5-(benzylmercapto)-1*H*-tetrazole is considerably decreased, which allows for preparation of an absolutely water-free activator solution.

5-(Benzylmercapto)-1*H*-tetrazole can easily be synthesised in the laboratory, which is another advantage in terms of saving costs for the otherwise rather expensive activator reagents. Typically, it is prepared from reaction of benzylthiocyanate with sodium azide and ammonium chloride as acidic catalyst. Protocols provided in the literature, however, yield only 27%^{5,7} or 48%⁸ of the product with the latter being raised to 63% by switching from 25 to 105 mmol scale.⁸ In order to find a strategy which allows for fast, inexpensive and efficient preparation of 5-(benzylmercapto)-1*H*-tetrazole we screened a variety of reaction conditions (Table 1).

The major problem is to dissolve both, the organic benzylthiocyanate and the inorganic salts (sodium azide and ammonium chloride). Thus, either DMF or an organic solvent/water mixture has to be used. DMF is difficult to remove after reaction and usually is present in trace amounts in the final product. Solvent/water mixtures also are disadvantageous, since benzylthiocyanate undergoes classical nucleophilic substitution with NaN₃ as a side reaction, that is even more facilitated with the azide anion in aqueous media.⁷ To circumvent these problems, initially we carried out the reaction with lithium azide, which is better soluble in organic solvents than its sodium analogue. For the same purpose ammonium acetate was used instead of ammonium chloride as a source of proton. The reaction was carried out over 6 h in absolute dioxan at elevated temperatures and delivered 5-(benzylmercapto)-1*H*-tetrazole in 65% yield (Table 1, line 1). Since the preparation of lithium azide is rather time consuming and expensive,¹⁰ we reinvestigated the synthesis of 5-(benzylmercapto)-1*H*-tetrazole with sodium azide and ammonium chloride as hydrazonic acid source. Under phase-transfer conditions as reported by LeBlanc and

Table 1. Screening of reaction conditions for the synthesis of 5-(benzylmercapto)-1*H*-tetrazole. All reactions were carried out at 0.1 mol scale

#	Solvent	Azide	Catalyst	T/°C	Time (h)	Yield (%)
1	Dioxan	LiN ₃	NH ₄ OAc	90	6	65
2	Toluene/H ₂ O (70:30)	NaN ₃	NH ₄ Cl, HDTMAB ^a	70	96	43
3	Dioxan/H ₂ O (80:20)	NaN ₃	NH ₄ Cl,	90	6	30
4	Dioxan/H ₂ O (80:20)	NaN ₃	NH ₄ Cl, raschig rings	90	6	62
5	Dioxan/H ₂ O (80:20)	NaN ₃	NH ₄ Cl, raschig rings	70	24	63
6	Dioxan/H ₂ O (80:20)	NaN ₃	NH ₄ Cl, raschig rings	70	96	72 ^b

^a Hexadecyltrimethylammonium bromide (phase-transfer catalyst).

^b Repeated preparation of 5-(benzylmercapto)-1*H*-tetrazole at 0.1 to 0.5 mol delivered the product independent of the reaction scale with 67 to 72% yield.

Jursic,⁸ at 0.1 mol scale we were able to produce the tetrazole derivative in 43% yield (Table 1, line 2). Using 80% aqueous dioxan as solvent we found, that sodium azide is still only poorly soluble, which might account for the observed low yield (Table 1, line 3). We carried out the reaction in the presence of Raschig rings in order to increase the reactivity of the azide by mechanical activation. As shown in the Table 1, yields are considerably improved (lines 4–6). Reaction over 6 h at 90°C delivered the product with 62% yield, that could be improved to 72% by applying longer reaction times and lower temperatures. The working up procedure involves repeated extraction of the aqueous reaction mixture with organic solvent.⁹ When the reaction was carried out under phase-transfer conditions,⁸ HDTMAB was found to disturb phase separation making the extraction less efficient. Thus, the protocol provided in this article is advantageous in terms of higher yields as well as easier handling.

In summary our results show, that 5-(benzylmercapto)-1*H*-tetrazole can be prepared from cheap reagents in a rather short time. Using 5-(benzylmercapto)-1*H*-tetrazole in RNA synthesis, coupling times are reduced and also the required amount of monomer building blocks as well as the activator is decreased compared to traditional methods. This is an important factor concerning costs and time for chemical preparation of oligoribonucleotides and should be of great interest to any chemist involved in RNA synthesis.

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- 74.5 g Benzylthiocyanate (0.5 mol), 40 g sodium azide (1.3 equiv.) and 69.5 g ammonium chloride (1.3 equiv.) were mixed with 300 ml of 80% aqueous dioxane. Raschig rings (glas, 4 mm, ø 4 mm) were added and the mixture was vigorously stirred for 96 h at 70°C with a strong magnetic stirrer. After cooling-down the solution was acidified to pH 2 with conc. HCl followed by addition of 100 ml water. The mixture was extracted three times with 200 ml CHCl₃. The combined organic layers were re-extracted with 3×100 ml 5% NaHCO₃, and the aqueous extracts again acidified to pH 2 with conc. HCl affording 65 g of 5-(benzylmercapto)-1*H*-tetrazole as a white powder (72% yield). The analytical data allowed for application of the product in oligonucleotide synthesis without further purification. The protocol for preparation of 5-(benzylmercapto)-1*H*-tetrazole in the presence of LiN₃ and anhydrous dioxan is analogous, but proceeds without Raschig rings. Analytical data: MS (EI): *m/z* 192.1 (*M*=192.24 g/mol). EA: theoretical (%) C, 49.98; H, 4.19; N, 29.14; S, 16.68. Found (±0.3%) C, 50.10; H, 4.06; N, 29.01; S, 16.60. ¹H NMR (CD₃CN): δ 4.26 (2H, s, CH₂), 7.05 (5H, m, aromatic); ¹³C NMR (CD₃CN): δ 38.2 (CH₂), 127.7/128.1/128.8 (CH), 137.0 (phenyl C), 160.6 (tetrazole).
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