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A Mild and Highly Selective *N*-Benzoylation of Cytosine and Adenine Bases in Nucleosides with *N*-Benzoyltetrazole[1]

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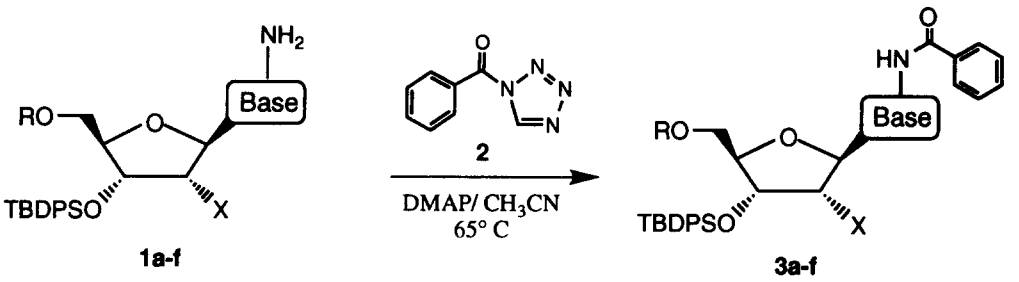
Abstract: *N*-Benzoyltetrazole has been developed as a mild and selective reagent for monobenzoylation of the exocyclic amino group in nucleic acid bases. Its usefulness is demonstrated by protection of adenine and cytosine bases, an important procedure in the nucleic acid chemistry field. © 1997 Elsevier Science Ltd.

The use of antisense oligonucleotides represents a powerful new strategy in the development of therapeutic agents which act by the selective inhibition of gene expression.[2] As modified oligonucleotides have become a major field of investigation for chemists,[3] thus suitable protection/deprotection methodologies for the synthesis of nucleoside monomers have become equally important.[4] In this respect, protection of exocyclic amino groups of nucleoside bases is one of the most important steps in the synthesis of oligonucleotides. One of the early methods of protecting these amino groups, developed by Khorana and co-workers,[5] consisted of peracylation, followed by selective deacylation of the hydroxyl groups with a base to obtain the desired *N*-acylated nucleosides. In 1982, Jones and co-workers[6] developed a "transient protection" method for the synthesis of *N*-acyl-2′-deoxynucleosides. This procedure requires temporary masking of the hydroxyl groups as trimethylsilyl ethers followed by reaction with excess benzoyl chloride or isobutyric anhydride in pyridine. An aqueous workup followed by base treatment provided the *N*-acylated nucleosides. Although this method has been the method of choice among nucleic acid chemists, there have been reports[7, 8] of formation of undesirable side products. Recently Sinha *et al*,[9] reported *N*-acylation of transiently protected adenine and cytidine nucleosides with freshly prepared *N*-acylimidazoles. This method also required the removal of silyl groups *via* hydrolysis. Although this method offers the advantage of monoacylation, the method requires absolutely dry reaction conditions and fresh preparation of the acylating reagent. Allyloxycarbonyl (AOC) protection of exocyclic amino groups in nucleosides has been reported by Hayakawa and Noyori[10] and was subsequently used by several other labs.[11] Interestingly, in this strategy the AOC group was activated by *N*-hydroxybenzotriazole or tetrazole, but the active acylating reagent was never isolated and characterized.

During the course of our research on backbone modified oligonucleotides we encountered some difficulties with the benzoylation of 3′-*O*-TBDPS-2′-*O*-Me-5′-formaldoxime adenosine (**1b**).[12] The traditional methods[5, 6] of benzoylation of **1b** furnished the product in a modest 55% yield along with some starting material and decomposition products.

Reaction Scheme:



This difficulty prompted us to look for a mild acylating reagent that would give the monoacylated product directly and in more acceptable yields. Herein we report the synthesis and application of *N*-benzoyltetrazole (**2**) as a new *N*-acylating reagent. Treatment of benzoyl chloride with 1*H*-tetrazole in the presence of triethylamine as an acid scavenger furnished **2** in excellent yield (86%). The product was easily crystallized from hexanes as colorless needles. The reagent **2** was stored in an amber bottle at 4° C under nitrogen and proved to be very stable for extended periods of time (~ 6 months). Treatment of nucleoside **1b** with two equivalents of **2** in the presence of one equivalent of 4-dimethylaminopyridine (DMAP) at 65° C in dry CH_3CN for 75 min furnished the desired N^6-benzoylated derivative **3b** in 75% isolated yield. Importantly, no dibenzoylation was observed under the described conditions. The identity of mono or bis benzoylated product was confirmed by comparison to the material obtained *via* Jones procedure.[6] This reaction was further extended and found to be applicable to several other modified nucleoside analogs. The results with monomeric nucleosides are summarized in the table.

Conditions and yields of acylation of **1a-f** to **3a-f**

Starting Material	Product	Base	R	X	Reaction Time (min.)	% Yield
1a	**3a**	Adenine	TBDPS	H	~ 75	80
1b	**3b**	Adenine	CH_2=N-	OCH_3	~ 75	75
1c	**3c**	Adenine	DMT	H	~ 90	72
1d	**3d**	5-Methyl cytosine	TBDPS	H	~ 15	80
1e	**3e**	Cytosine	DMT	H	~ 15	85
1f	**3f**	5-Methyl cytosine	CH_2=N-	OCH_3	~ 15	90
1g	**3g**	5-Methyl cytosine	DMT	H	~ 15	80
1h	**3h**	5-Methyl cytosine	DMT	$O(CH_2)_2OCH_3$	~ 15	90

Interestingly, when an equimolar mixture of protected 2′-deoxyadenosine **1a** and 2′-deoxycytidine **1e** was treated with two equivalents of **2** and one equivalent of DMAP in CH_3CN for 10 min at 65° C only monobenzoylation of cytidine was observed, no benzoylation was detected for the adenine residue in **1a**. When an additional two equivalents of **2** along with one equivalent of DMAP was added to the above mixture at the same

temperature, **1a** was completely converted to the corresponding monobenzoylated product and no bis-benzoylation of **1e** was detected. This selectivity may be particularly useful in protecting mixed dimers required for backbone modified antisense oligonucleotides[3b] containing both cytidine and adenine residues. Narang and co-workers[13] have made a similar observation when they perbenzoylated nucleoside derivatives with a mixture of benzoyl chloride and 1*H*-tetrazole, possibly forming *N*-benzoyltetrazole *in situ*.

It is noteworthy to mention here that when compounds **1a** and **1d** were treated with **2** in absence of DMAP, the corresponding monoacylated nucleosides were obtained in excellent yields. This observation suggests a possible mechanism of reaction in which direct nucleophilic attack of the exocyclic amino group on **2** occurs. **1c** and **1g** react similarly with **2** in the absense of DMAP, although 5-10% detritylation was observed in these reactions. This could possibly be explained by the acidic nature of 1 *H*-tetrazole (p*K*a = 4.89)[14] and any benzoic acid (p*K*a = 4.2) liberated during the reaction. Therefore, DMAP works as a buffer in the above reactions. The structures of all the compounds described in this study were confirmed by ^{1}H NMR and thin layer chromatography with authentic samples. We have also accomplished the successful mono benzoylation of MMI dimers (for definition of MMI dimer, see refrence 12) containing mixed bases (C*G and C*A). These results will be reported elsewhere.

In summary, *N*-benzoyltetrazole has been synthesized[15] in excellent yield and isolated for the first time as crytalline solid which is very stable for several months when stored dry at 4° C. We have demonstrated that the selective monobenzoylation of adenine and cytosine residues in nucleosides is possible with this reagent. We believe that this reagent will be an important addition to the already existing methods of protection of the exocyclic amino groups in nucleosides and nucleotides. Other *N*-acyltetrazolides, for example, *p*-toluylthionocarbonyl-tetrazole, benzoyloxohydroxybenzotriazole and isobutyryltetrazole have also been prepared and study of their possible use is in progress.

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15. Preparation and properties of **2**: To a stirring suspension of 1*H*-tetrazole (7.0 g, 0.1 mol) and triethylamine (15 ml, 0.12 mol) in anhydrous THF (300 ml) at 0° C (bath temperature) was added benzoyl chloride (99%, 12.8 ml, 0.11 mol) slowly over 30 min. The mixture was stirred for an additional 45 min. The resulting precipated solid (triethylammonium hydrochloride) was removed by filtration through a pad of celite 545. The solvent was removed under reduced pressure keeping the bath temperature below 30° C. The residual oil was dissolved in hexanes to furnish *N*-benzoyltetrazole as colorless crystals which were collected by filtration, washed with hexanes (2 x 15 ml) and dried under vacuum for ~15 hr at room temperature. Yield: 15 g, 86%; m.p. 56-58° C; ^{1}H NMR, ($CDCl_3$) δ 9.43 (s, 1H), 8.26-8.22 (d, J = 2H), 7.79-7.54 (m, 3H); ^{13}C NMR, ($CDCl_3$) δ 162.9, 143.7, 136.3, 132.7, 129.7 and 129.5. HRMS: (FAB) for $C_8H_7N_4O$ (MH^+) 175.0620, found 175.0627.

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