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Novel thermolabile protecting groups with higher stability at ambient temperature

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ABSTRACT

Novel thermolabile hydroxyl protecting groups of increased thermostability are proposed. The stability of these groups at different temperatures ranges has been determined. The 2-pyridyl-*N*-(2,4-difluorobenzyl)aminoethyl unit was selected as stable at ambient temperature and very labile at increased temperature. The half-life times for the best groups were established. Application of this chemistry to the protection of different hydroxyl groups of thymidine was also demonstrated.

With the development of further applications of synthetic biomolecules, for example, nucleic acids, peptides, etc., rapid progress in devising new systems for protecting reactive sites of molecules is being made. This trend focuses on the development of groups whose removal may be carried out in vivo. However, apart from the protective function most of the popular protecting groups often find multi-functional uses.[1]

Very useful protecting groups have been proposed, which can be removed only by increasing the temperature. Due to this property, they have been termed thermolabile protecting groups (TPG). The first thermal deprotection method was presented for the protection of a phosphate during the synthesis of nucleic acids.[2,3] Nucleophilic intramolecular thermocyclization was the driving force for removal of TPGs and the kinetics depend closely on the temperature. This reaction can be conducted successfully in aqueous environment at pH 7 as well as in organic/aqueous solvents. 2-(*N*-Formyl-*N*-methyl)aminoethyl,[4] 3-(*N*-*tert*-butylcarboxamido)-1-propyl,[5] 3-(2-pyridyl)-1-propyl,[6] and 4-methylthio-1-butyl[7] were tested as effective protecting groups for phosphate. Among TPGs, the 4-oxopentyl group deserves special attention as it enables effective and economical protection of phosphate and thiophosphate centers. This group can be removed by intramolecular cyclodeesterification induced by heating or using either pressurized gaseous amines, or concentrated ammonium hydroxide.[8] However, none of these groups has been used effectively to protect a hydroxyl moiety due to the high durability of the appropriate carbonate derivatives used. Also the 2,2,5,5-tetramethylpyrrolidin-3-one-1-sulfinyl group exhibited near optimal properties for 5′-hydroxyl protection by virtue of the mildness of its cleavage conditions.[9]

Recently, the introduction of the nucleophilic 2-aminopyridyl system[10] has made it possible to develop a new class of thermolabile groups which are useful for protection of the hydroxyl functional group.[11] Their usefulness as phosphate protecting groups increased after a 'click–clack' concept was devised, a method which temporarily blocks the thermolabile properties of TPG.[12]

2-[*N*-Methyl-*N*-(2-pyridyl)]amino-1-phenylethanol (**1**) was selected as a particularly promising precursor of thermolytic carbonate for hydroxyl group protection.[13] It was characterized by a total unblocking time of approximately 15 min at 90 °C. However, partial removal of this group at ambient temperature has been observed (10%, 24 hours in acetonitrile). This limits considerably the application of protecting groups for multi-stage syntheses (e.g., during the synthesis of nucleic acids).[14] Structural and crystallographic analysis[15] showed that its isomer **2** could be a good candidate for thermolytic carbonates (Scheme 1). These findings prompted further investigation of *N*-pyridyl-*N*-benzylaminoethyl carbonates for protecting hydroxyl functions. The application of isomer **2** as a precursor of TPG brings the following advantages: (i) eliminates the chiral center at the carbon atom; (ii) increases the nucleophilicity of the pyridine ring, thus affecting the unblocking rate; (iii) reduces the cost of the TPG group synthesis.

In an effort to find the optimum medium between shortening the time of deprotection and increasing the stability of the thermolabile protecting group, new TPGs based on *N*-pyridyl-*N*-benzylaminoethyl carbonates were investigated (Scheme 1). Their precursor, *N*-pyridyl-*N*-benzylaminoethanol, was prepared from

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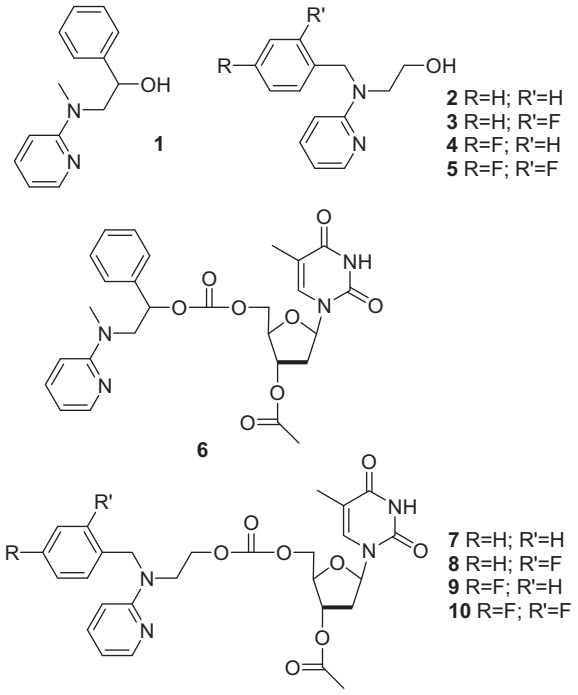


Scheme 1. Structures of the precursors of thermolabile protecting groups and their active carbonates.

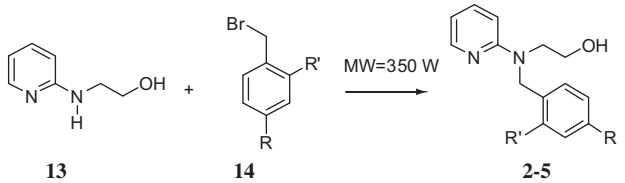
commercially available reagents via nucleophilic substitution as presented in Scheme 2.[16] The synthesis of amino alcohols—TPG precursors—involved reaction of 2-pyridylaminoethanol (**13**), by means of microwave irradiation, with fluoro-substituted benzyl bromides **14**. The MW assistance reduced the reaction time and increased the yield of the total process.

Herein is reported the preparation and characterization of novel precursors **2**–**5** and their application for the protection of hydroxyl groups in nucleosides to give thermolabile carbonates **7**–**10** (Scheme 1). The deprotection kinetics of these groups for the model 3′-*O*-acetylthymidine and 5′-*O*-DMT thymidine are presented.

A characteristic feature of these groups is the presence of a benzyl group at the exocyclic amine. Preliminary experiments have shown that fluorine atoms on the aromatic ring stabilize thermal carbonates.

In particular, the *para* and *ortho* positions on the benzyl ring have a significant impact on the stability of TPGs in acetonitrile. An attempt to synthesize a TPG containing methyl groups, instead of fluorine, was unsuccessful.

Additionally, the introduction of two fluorine atoms on the benzyl ring may generate additional types of interactions due to a possibility of F···H–C bonds. For compound **5** in which the fluorine atoms are located at the *ortho* and *para* positions the NOESY-type interactions in the two dimensional ^{1}H–^{19}F NMR HOESY spectrum at 20 °C were observed between the *ortho* fluorine atom and



Scheme 2. Synthesis of the TPGs precursors.

hydrogen atom of the alkyl chain (Fig. 1). This kind of interaction will move the benzyl group toward the alkyl chain, limiting the thermal deprotection reaction due to the inaccessibility of the electrophilic center. Probably, in the case of compounds **8** and **9** with shorter half-life times at 20 °C, there is no interaction between the benzyl ring and the chain.

A study of the kinetics of the thermal unblocking reaction was performed using model 3′-*O*-acetylthymidine where the 5′-OH function was protected by the TPG. The presence of a 3′-*O*-acetyl group indicates that the deprotection process does not follow the ester hydrolysis mechanism. The protection of a free 5′-hydroxyl group consists of a two-step reaction of carbonyldiimidazole with the amino alcohol/TGP precursor and a hydroxyl group, which results in compounds **7**–**10**. The final products were purified by silica gel chromatography at 5 °C and characterized by ^{1}H NMR spectroscopy and high-resolution mass spectrometry. Removal of the protecting group (Scheme 3) was conducted in acetonitrile/aqueous phosphate buffer mixture at two different temperatures, 90 and 20 °C. It is proposed that the half-life times can be used for evaluation of the thermolability of TPGs for a given group under defined conditions.[17] The obtained values of half-life times are presented in Table 1, and the deprotection curve is shown in Figure 2.

From the data presented it can be concluded that fluorine derivatives have higher half-life times at 90 °C. Compounds **7** and **8** are characterized by shorter half-life times at 90 °C. The situation is quite different when the stability of these groups at ambient temperature is analyzed. Compounds **8** and **9**, which have one fluorine atom, showed lower stability of the carbonate under these conditions. However, **10** and in particular **15**, have increased thermal stability, allowing their free manipulation. These compounds also have longer half-life times at 90 °C.

All the analyzed *N*-benzyl derivatives, compared to previously published compound **6**, are characterized by increased stability at ambient temperature. It is known from the literature[10] that 10% deprotection of compound **6** occurs in anhydrous acetonitrile at ambient temperature over 24 h. For compounds **7**, **8,** and **9** the thermal deprotection level under these conditions did not exceed 7%, and for compound **10** was 4%.

These characteristics may be due to restricted rotation in certain parts of the molecule. This makes adoption of a convenient conformation for intramolecular cyclization difficult. It seems that the presence of the benzyl ring is a significant factor in the thermal control of the molecule at lower temperatures.

The usefulness of a protecting group increases when it is possible to monitor its blocking and unblocking (Fig. 3). The blocked form of *N*-benzyl TPG **7**–**10** has an additional diagnostic absorption maximum at 308 nm in the UV–vis spectrum. In the UV–vis spectrum for bicyclic compound **12**, a shift of the absorption band occurs to a new maximum at 337 nm. Measurement of the absorption intensity at 337 nm can be used as a diagnostic element to determine the level of TPG unblocking (Fig. 4).

It can be concluded that the TPG based on precursor **5** may have practical application for the protection of a hydroxyl function. To demonstrate the usefulness of TPG in protecting a secondary hydroxyl, 5′-*O*-DMT thymidine was selected as a model compound. Protection of this secondary hydroxyl group was performed under the same conditions as in the previous example. The results of the kinetic experiments showed that such a protecting group had a higher stability (higher half-life time—Table 1). In addition, it was shown that removal of a thermolabile protecting group does not affect the stability of the acid–labile DMT group. This finding has extended the scope of applications for TPGs in chemical synthesis of biomolecules where a DMT group is popular.

Compounds **12** and **17** are products of intramolecular cyclization (Scheme 3). Analysis using NMR spectroscopy showed that a bicyclic thermocyclization product was formed. This proves that

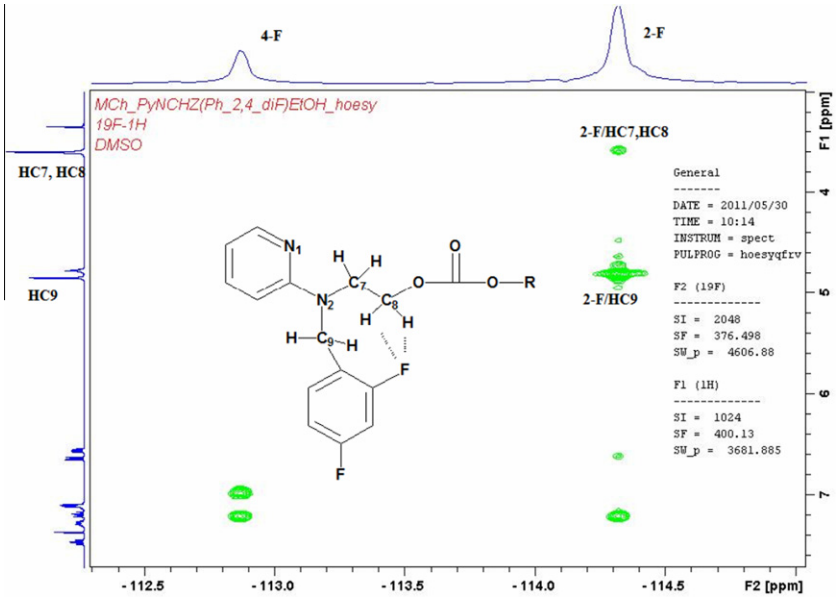
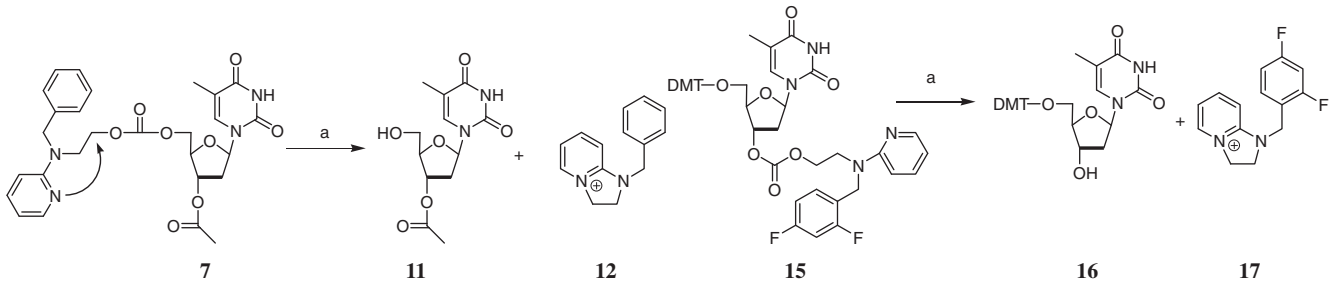


Figure 1. Two dimensional $^{1}H–^{19}F$ HOESY spectra for **5**. Interactions between the F and H atoms are observed as strong signals between benzyl and weaker between methylene hydrogens.



a = MeCN/buffer PBS pH=7.4; 90 °C; 10 min.

Scheme 3. Deprotection of TPG in protected nucleosides, from a primary hydroxyl group in **7** and from a secondary hydroxyl in **15**.

Table 1
Half-life times for *N*-pyridyl-*N*-benzylaminoethyl carbonates[a]

	7	8	9	10	15
90 °C (min)	1.8	1.8	2.1	2.7	2.5
MeCN, 20 °C (h)	210	145	170	245	288
MeCN/phosphate buffer, 20 °C (h)	29	26	30	58	57

[a] Calculations are based on RP C-18 HPLC analysis.

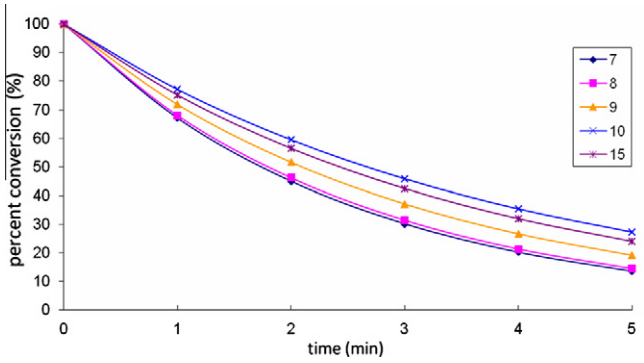


Figure 2. Modeled curves for the deprotection of TPGs in buffer pH 7.4/MeCN at 90 °C.

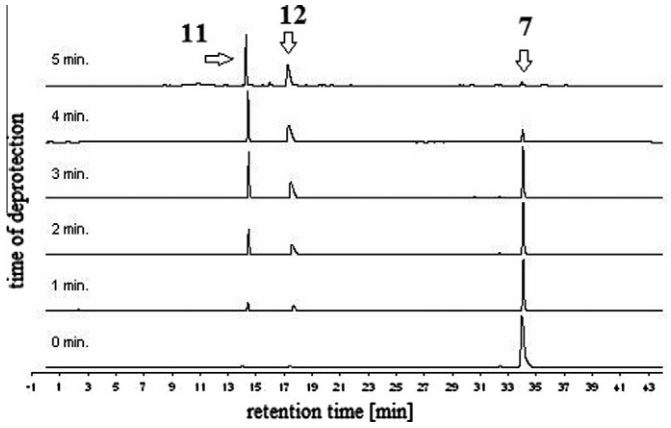


Figure 3. RP-HPLC profiles for the thermodeprotection of **7**. Analyses were performed using a 3 μm Luna C(18)2 100 Å column (15 cm × 4.6 mm) according to the following conditions: starting from 0.01 M triethylammonium acetate pH 7.0, a linear gradient of 1% MeCN/min was pumped through at a flow rate of 0.8 mL/min.

intramolecular cyclization occurs according to a mechanism of nucleophilic attack at the carbon atom adjacent to the carbonyl group.

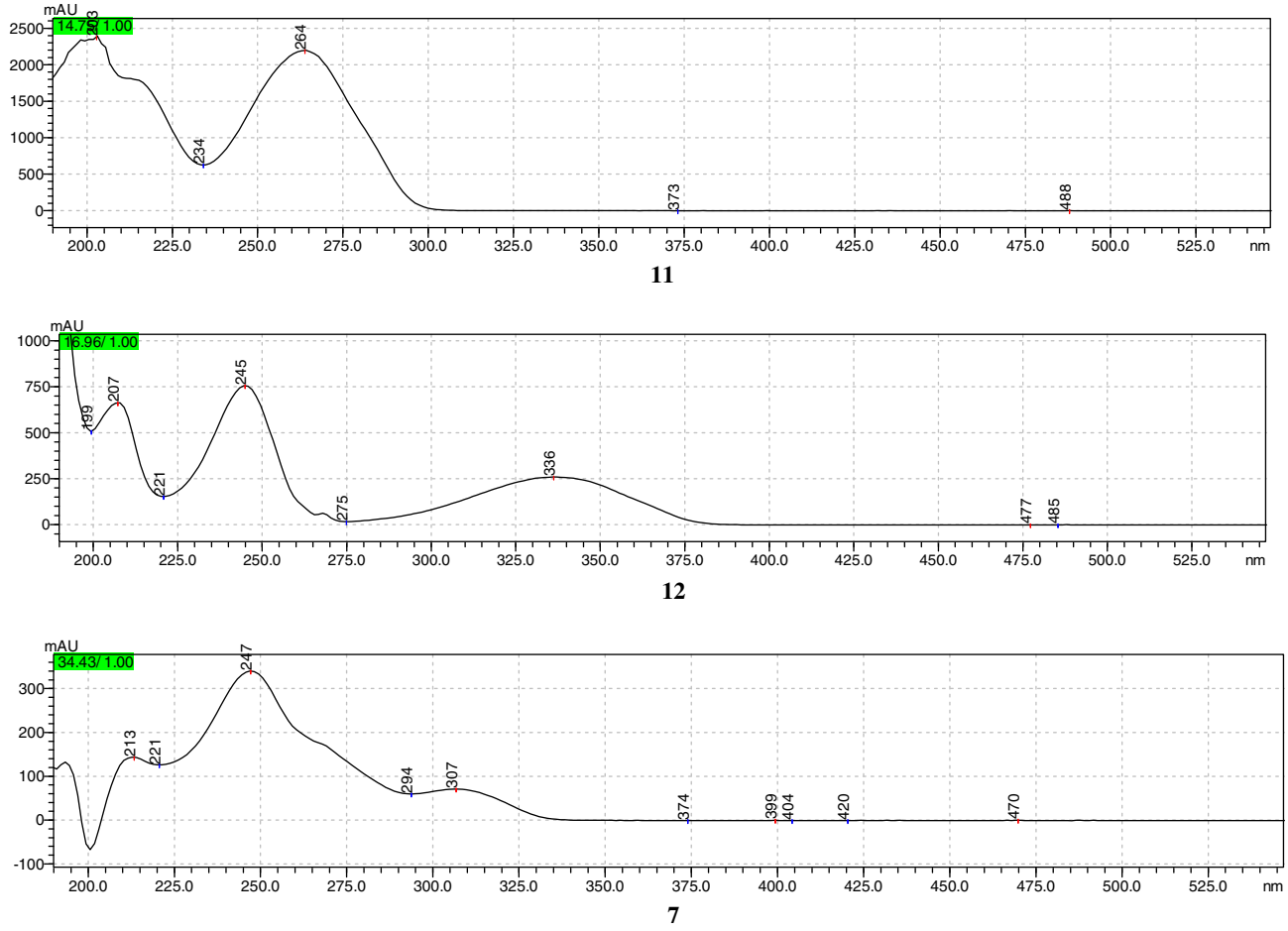


Figure 4. UV–vis profiles of each of the detected peaks. Analyses were performed using a UFLC Shimadzu System equipment with a diode array UV–vis detector.

In summary, new and useful thermolabile protecting groups with increased stability at ambient temperature have been presented. The half-life times for these groups do not exceed 2.7 min. The study also shows that these compounds are stable in anhydrous acetonitrile, but during long term storage in solution these groups should be kept at 5 °C. It is planned to determine the factors influencing the higher stability of 2-pyridyl protecting groups in further research.

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Supplementary data

Supplementary data (details on the experimental procedures, NMR and mass spectra as well as calculations of half-life times and RP-HPLC profiles of the thermodeprotection of **15**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.11.122.

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