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2-Pyridinyl-*N*-(2,4-difluorobenzyl)aminoethyl Group As Thermocontrolled Implement for Protection of Carboxylic Acids

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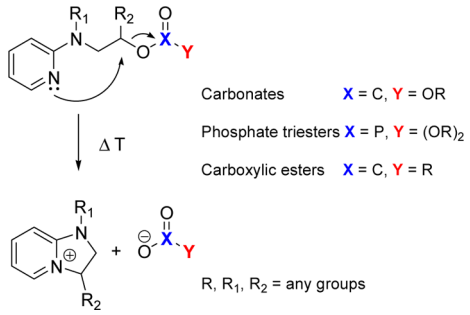
Supporting Information

ABSTRACT: A thermolabile protecting group strategy for carboxylic acids is expanded. Thermosensitive esters are readily prepared using a known procedure, and their stability under neutral condition is investigated. Effective thermolytic deprotection initiated only by temperature for different carboxylic acids is demonstrated, and the compatibility of a thermolytic protecting group with acidic and basic protecting groups in an orthogonal protection strategy is also presented. This study showed interesting correlations between the pK_a of acids and the deprotection rate of their well-protected moieties.

Chemical strategies for synthesizing nature-based biopolymers (peptides,[1] nucleic acids,[2] or polysaccharides[3]) still require a combination of various protecting groups to mask naturally occurring functions.[4] These protecting groups are stable during biopolymer synthesis or transformation but should be removable selectively when required. This selectivity has recently been carried out by applying mild conditions manifested in enzymatic or thermolabile approaches. Thermolabile Protecting Groups (TPGs) are a modern concept where deprotection takes place in neutral conditions only by increasing temperature.[5] This approach was successfully applied in phosphate,[6] hydroxyl,[7] and amine[8] protection, which assumes universality of the thermolytic approach. In order to demonstrate extension of the mentioned approach, we explored it for other functional groups. Despite the fact that carboxyl groups are less present in biopolymers (some peptides or oligosaccharides, lipids), their dual nature (electrophilicity of a carbonyl group and nucleophilicity of a hydroxyl group) requires a specific strategy especially when they must remain protected until the very last step. In practice, the carboxyl group is most frequently protected by transforming it into an ester form.[9] For more popular applications, tris(triethylsilyl)silyl,[10] *tert*-butyl,[11] benzyl,[12] 9-fluorenylmethyl,[13] 1,3-dioxanes,[14] ethyl,[15] 2-trimethylsilylethyl,[16] and 2,2,2-trichloroethyl are applied.[17,18] Additionally, selection of the proper strategy for protecting reactive functions is mainly based on customized sets of protective groups, when it is necessary to remove one protecting group without affecting others. Therefore, an orthogonal protection strategy assumes removal of acid-labile protecting groups without affecting base- or heat-labile ones which remain stable under these conditions.[19]

2-Pyridinyl Thermolabile Protecting Groups (2-PyTPGs) have previously been applied successfully as carbonates in hydroxyl protection, as phosphate esters in phosphate protection,[20] and in polymerization of poly(methacrylic acid).[21] Their deprotection mechanism via specific intramolecular cyclization[22] is based on nucleophilic pyridine nitrogen attacks on a carbon atom and formation of an unstable carbonic ion or phosphate anion (one negative charge) (Scheme 1). Therefore, we can hypothesize that

Scheme 1. General Mechanism for Thermocyclization and Ion Formation



thermocyclization might occur with a high yield only when monoanionic acid is formed.[23] In the context of carboxylic ions which can have one negative charge, we can postulate that the 2-pyridinylaminoethyl ester of carboxylic acid will undergo thermocyclization, thus forming such an ion. In other words, formation of an ion with one negative charge may be a driving force of 2-PyTPG thermolytic removal from carbonates, phosphate triesters, or carboxylic esters.

The present paper is the first report on using a 2-pyridinyl-*N*-(2,4-difluorobenzyl)aminoethyl (PydFBz) thermolabile protecting group for carboxylic acids protection which can be selectively removed under neutral conditions.

We performed the study of their thermal stability in neutral pH at different temperatures in relation to the acidic character of examined acids. Additionally, we investigated the orthogonality of PydFBz TPG with other protecting groups: acid-labile bis(4-methoxyphenyl)phenylmethyl (DMT) and base-labile

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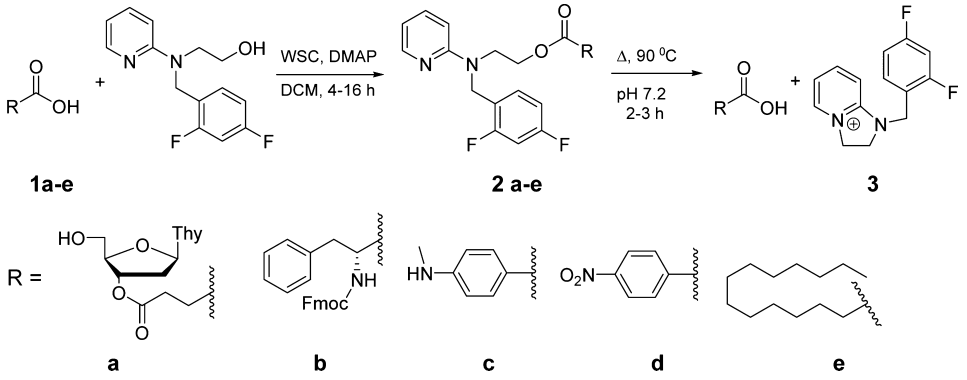
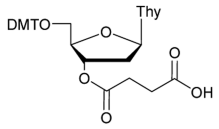
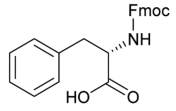
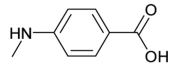
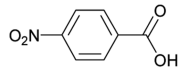
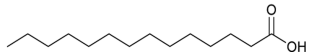
Scheme 2. Structure of Investigated Acids and Thermal Deprotection of PydFBzTPG from Their Esters



fluorenylmethyloxycarbonyl (FMOC) as typical representatives in oligonucleotide[24] and peptide synthesis,[25] respectively. 2-Pyridinyl-*N*-(2,4-difluorobenzyl)aminoethyl esters (**2**) were prepared by conventional methods, from the corresponding carboxylic acid (**1**) and 2-pyridinyl-*N*-(2,4-difluorobenzyl)-aminoethanol (versatile TPG precursor) under mild basic catalysis (DMAP). Effective transformation into a protected carboxyl moiety **2** was carried out in the presence of a condensing agent, i.e. WSC or DIC (Scheme 2).

Because we postulated that the thermal stability of TPG depends on the ionic strength (i.e., concentration of electrical charge) of the acid departing anion, we selected carboxylic acids according to the different pK_a values (Table 1). The acids represent different classes whose properties vary in the structure (aliphatic **1e** or aromatic **1c**, **1d**), electron distribution (electron-donating group **1c**, electron-withdrawing group **1d**), or their functionality as biologically important compounds, i.e. nucleoside **1a** and amino acid **1b**. This juxtaposition allows us to observe a difference in their thermal deprotection rate and could be helpful for designing more comprehensive 2PyTPG.

Table 1. Carboxylic Acids Used in Thermal Protection

entry	carboxylic acid	pKa	isolated yield (%) 2a-e
1a		4.21[b]	48
1b		2.58[a]	84
1c		5.04[b]	92
1d		3.44[b]	90
1e		4.9[b]	98

[a]https://www.anaspec.com/html/pK_n_pl_Values_of_AminoAcids.html. [b]Dean, J.A. Handbook of Organic Chemistry; New York, NY: McGraw-Hill Book Co.: 1987; pp 8−50. Fmoc: fluorenylmethyloxycarbonyl. DMT: bis(4-methoxyphenyl) phenylmethyl. Thy: thymine.

Well-protected carboxylic acids **2a−e** were studied to determine their thermolytic properties in two ways: (i) determining the stability of **2** under different conditions and (ii) finding optimal conditions for fast and selective deprotection.

During the kinetic studies, we observed the formation of only two main products on HPLC chromatograms which were identified as recovered carboxylic acids **1**, and 1-(2,4-difluorobenzyl)-2,3-dihydro-1*H*-imidazo[1,2-*a*]pyridine-4-ium **3** with a characteristic absorption band at maximum 337 nm.[26] The presence of the bicyclic product **3** confirms the mechanism known from carbonate thermocyclization.[27] Table 2 shows the data of the deprotection rate for protected carboxyl moieties **2a−e** which are presented in time position (1 and 2 h heating) and include room (25 °C), lower (60 °C), and higher (90 °C) temperatures.

Table 2. Percentage of Thermodeprotection of 2a−e at Different Temperatures and Times

	2 h, 25 °C	2 h, 60 °C	1 h, 90 °C	2 h, 90 °C
2a	0	8	54	78
2b	3.5	29	100	100
2c	0	1.9	14	24
2d	3.2	31	98	100
2e	0	22	18	45

Under neutral conditions and at room temperature, the 2-pyridinyl-*N*-2,4-difluorobenzylaminoethyl group is totally stable in aqueous solution or organic solvents. We demonstrated that total deprotection of the 2-pyridinyl-*N*-2,4-difluorobenzyl-aminoethyl group is possible and effective in an aqueous solution of **2** through simply raising the temperature. The deprotection rate is a function of temperature, but this dependence is not linear. We noticed that deprotection significantly accelerates over 60 °C. However, the relation between thermal deprotection rate and time at 90 °C is constant (Table 2).

We also found a strong relation between pK_a values of carboxylic acids **1** and the deprotection rate of their protected moieties **2**. For the well-protected acids, whose pK_a was below 4, thermal deprotection was quite fast and very effective. However, for less acidic carboxylic acids total removal of the 2-pyridinyl-*N*-(2,4-difluorobenzyl)aminoethyl group from esters may be difficult, even at high temperatures (Table 2). We assumed that the deprotection rate is directly proportional to

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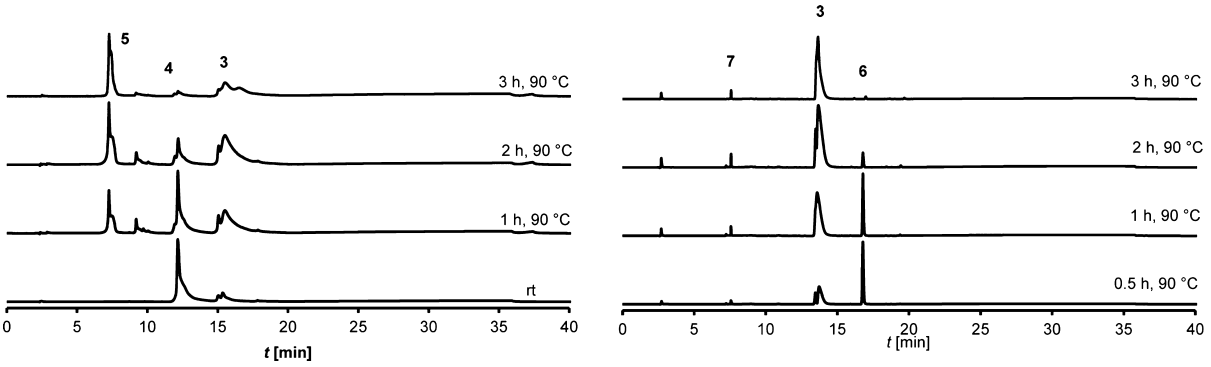
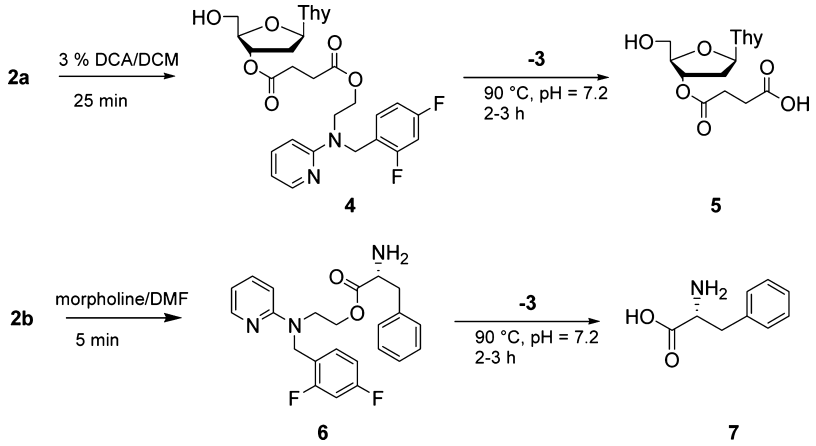


Figure 1. HPLC analysis of **2a** (left) and **2b** (right) shows the compatibility of TPGs with acidic and basic protecting groups.

Scheme 3. Compatibility of PydFBz TPG with Acid- and Base-Labile Groups



pK_a. This dependence is as follows: the lower the pK_a, the more labile the protecting group is under thermolytic conditions. As we expected, the formation of a more stable carboxylic anion during thermocyclization generates a better leaving group which is the driving force of the deprotection process. Additionally, we observed that storing esters **2** in anhydrous organic solvent within 5 days did not result in significant deprotection, particularly for acids with a pK_a over 4. Our investigations have led to the following conclusions: (i) thermal stability depends on the pK_a of carboxylic acids; (ii) a lower pK_a increases the deprotection rate; (iii) the PydFBz group is not suitable as a protecting group for acids with a pK_a over 4, because it remains stable for several hours; and (iv) 2-pyridinyl protecting groups could be suitable for the orthogonal protection strategy that uses acid and basic labile protecting groups. Since the deprotection mechanism is based on intramolecular cyclization, TPGs could be selectively removed in the presence of other classes of protecting groups.

Therefore, deprotection of thermolabile groups in neutral conditions is a good reason for testing them in an orthogonal protection system for nucleic acids or peptide synthesis. We demonstrated that the acid-labile bis(4-methoxyphenyl)phenylmethyl (DMT) and base-labile fluorenylmethyloxycarbonyl (FMOC) group were successfully removed, while the PydFBz TPG remained intact (Figure 1). The performed HPLC analysis revealed that a strong acid (3% TFA/DCM, rt, 5 min) and basic conditions (morpholine/DMF, rt, 1 h) had no influence on the stability of TPG protected nucleosides and amino acid at ambient temperature. Moreover, after removal of acid- and base-labile groups, full thermodeprotection of **2a**−**b** with release of the unmasked molecule and bicyclic product is still possible within 3 h (Scheme 3). These observations show that the protecting acid-labile (DMT), base-labile (FMOC), and heat-labile (PydFBzTPG) groups are orthogonal to one another. The advantage of this strategy is that we can successfully remove TPG and that PydFBz could be a suitable participating group for biopolymer synthesis.

We have shown that the 2-pyridinyl-*N*-(2,4-difluorobenzyl)-aminoethyl group is a proper, quantitatively removable protecting group for the acids with a pK_a below 5 under neutral conditions. Selective removal of the PydFBz group can be also accomplished in the presence of the two most common biopolymer protecting groups (DMT and Fmoc).

Moreover, we have confirmed that the deprotection mechanism via intramolecular cyclization of 2-pyridinyl-*N*-(2,4-difluorobenzyl)aminoethyl ester is the same as the mechanism investigated on thermolytic carbonates. Due to its advantageous properties such as ease of introduction, selective removal, and orthogonality, the PydFBz is foreseen as a valuable tool for the protection of carboxyl groups.

Thorough recognition of structural dependencies of individual TPGs and examination of the deprotection mechanism will allow us to precisely design a protecting group for concrete application. We are also planning to study the stability of thermolitic esters in a wide range of temperatures and pH's. In the near future we will be testing the chemical synthesis of peptides using thermolytically protected carboxylic acids.

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■ ASSOCIATED CONTENT

Supporting Information

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General synthetic procedures, HPLC analysis at different temperatures and times, and NMR and HRMS data for all discussed compounds (PDF)

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Notes

The authors declare no competing financial interest.

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