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A new protecting group for the exocyclic amino groups of nucleosides

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Abstract—A new protecting group has been developed for the exocyclic amino groups of nucleosides that occur in DNA. 3-Phenyl-[{N-(2-trimethylsilyl-ethoxycarbonyl)-2-amino}]-propanoic acid used as the protective agent.

Currently modified synthetic DNA, required for DNA damage and mutagenic studies[1] is obtained essentially in one of two ways, either by a presynthetic or a postsynthetic strategy. Nevertheless, at present, most modified DNA is prepared by the former method in which the modified base is pre-synthesized in a protected form[2] and then introduced into the oligomeric chain either by solution-based methods or by an automated resin-based procedure.[3] In a few instances postsynthetic strategy[4] was used successfully whereas more rarely a combination of both of these strategies has been employed. The latter approach, for example, was adopted to incorporate both the base-labile acrolein adduct of deoxyguanosine[5a] and the abasic site[5b] into DNA.

In our initial studies towards the synthesis of modified DNA we developed a protecting group, **1** (Fig. 1), which would be resistant to the normal basic conditions used in the deprotection of synthetic DNA.[6] Although this strategy was highly successful in cases where the oligomer contained only two dG residues, its incomplete resistance to alkali left it wanting in examples where more than two dG residues were present. An alternative strategy that might be used to obtain a site-specifically modified DNA by a postsynthetic strategy is to deprotect site-specifically an exocyclic amino group in a nucleoside while the DNA is still attached to resin. The deprotected base because of its greater reactivity than the other protected bases will react with a suitable electrophilic reagent to give the desired modified nucleoside residue. Unfortunately few amino protective groups can be removed selectively while the DNA is still attached to resin. The amino protective groups that are currently in use in the deoxynucleoside series are base labile and are uniformly removed by treatment either with ammonium hydroxide at 55 °C for 16 h or methylamine[7] at 23 °C. This allows no discrimination amongst the amino groups available for site-specific modification.

3-(2-Aminoaryl)-propionamides are a class of compounds, which disintegrate in a facile manner by internal cyclization to the corresponding free amino residue and a lactam. Because of their spontaneous internal cyclization[8] the 2-amino group in 3-(2-aminoaryl)-propionamides is always generated from the corresponding 3-(2-nitroaryl)-propionamides. The nitro group serves as a masked amino group until it's reduced to the corresponding amino group either under neutral,[6] acidic,[9a] basic,[9b] reductive hydrogenation[9c] or bioreductive[9d] conditions. For the first time, however, we were able to devise a method to introduce the above 2-amino

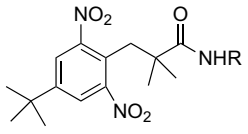

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Figure 1.

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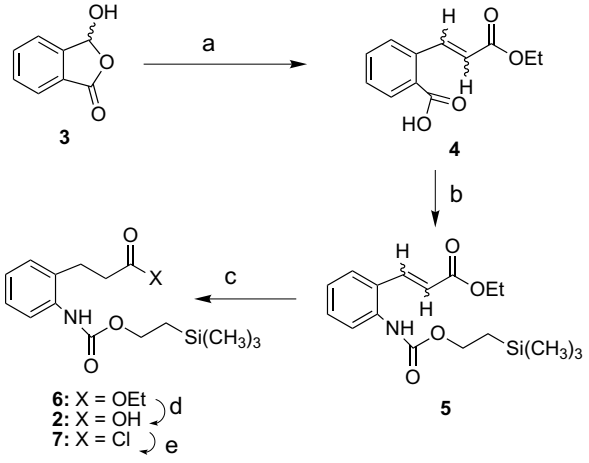
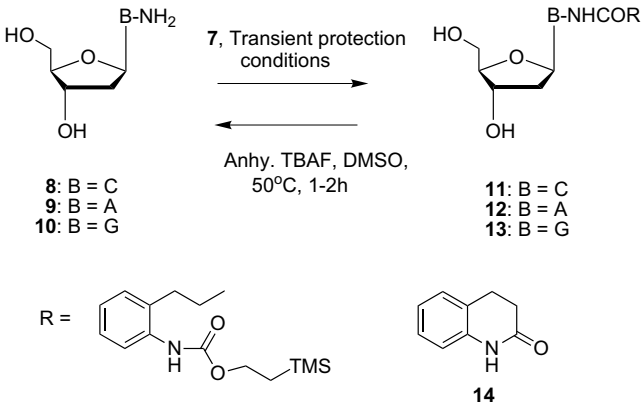
group in a carbamate form, in situ, capitalizing on Curtius rearrangement of the corresponding benzoyl azide in presence of the desired alcohol.

Herein we describe the synthesis of 3-phenyl-[{N-(2-trimethylsilyl-ethoxycarbonyl)-2-amino}]-propanoic acid (PTAP, **2**), which has several salient characteristics, namely, (i) it is readily prepared in four steps in overall high yield (Scheme 1); (ii) using this agent the three deoxynucleosides dC, dA and dG were derivatized under transient protection conditions in a facile manner and (iii) it is deprotected by anhydrous terabutlyammonium fluoride (TBAF) in a cascade of spontaneous steps that culminate in the free nucleoside and co-generation of lactam **14** (Scheme 2). Only one other example of the use of this type of protective group has been reported to protect specifically the exocyclic amino groups of DNA nucleosides, that of by Van Boom and coworkers[10] in which the 2-(*t*-butylphenylsilyloxymethyl)-benzoyl group was used. This was subsequently removed by fluoride ion. To our knowledge this kind of strategy using protected 3-(2-aminoaryl)-propionamides has never been applied in the protection of exocyclic amino groups of DNA nucleosides.

The desired acid **2** was prepared from the readily available and inexpensive 2-carboxybenzaldehyde, **3**. This was treated with commercially available (carbethoxymethylene)-triphenylphosphorane first at 0 °C and thereafter with stirring at 23 °C for 4 h to obtain the geometrically isomeric mixture of the ethyl esters **4**. The crucial carbamate **5** was obtained in a one-pot Curtius rearrangement[11] from **4** by treatment with diphenylphosphoryl azide (DPPA), NEt_3 and 2-trimethylsilylethanol in dioxane at 80–90 °C for 5 h, in 89% yield after silica gel chromatography. Catalytic hydrogenation (H_2, 10% Pd/C, 50 psi) of the product mixture **5** then gave the corresponding saturated ester **6**, which on mild alkaline hydrolysis with LiOH (10 equiv, DME:H_2O:: 1:2) at 23 °C for 5 h afforded the target acid **2** in 90% yield.



Scheme 1. Reagents and conditions: (a) $Ph_3P{=}CHCO_2Et$ (1.0 equiv), THF, 4 h, 23 °C; (b) DPPA, NEt_3, dioxane, 85 °C, 5 h; (c) H_2 (50 psi), 10% Pd/C (w/w), EtOH, 23 °C, 4 h; (d) LiOH (10.0 equiv), DME–H_2O (1:1), 23 °C, 5 h; (e) $SOCl_2$, (1.0 equiv), NEt_3, (1.5 equiv), 23 °C, 2 h.



Scheme 2.

The acid chloride derivative **7** of the acid **2** was prepared by means of $SOCl_2$ (1 equiv, 23 °C, 2 h) in the presence of NEt_3 (1 equiv) in dichloromethane. The acid chloride was then used to derivatise[12] the nucleosides **8**, **9** and **10** as the corresponding N-acyl derivatives[13] **11**, **12** and **13** (yields: 60–65%) by following the standard transient protection method.[14] Interestingly, unlike in the other reported methods[10] we didn't observe any di-substituted derivatives with dA and dG.

The deprotection[15] of nucleosides **11**, **12** and **13** was carried out by treatment with anhydrous TBAF[16] (3 equiv) in DMSO for 2 h at 50 °C. This cleanly regenerated the corresponding nucleosides **8**, **9** and **10** in excellent yields accompanied by the lactam **14**. The cyclization of 2-aminoarylamide released upon deprotection of amino group was instantaneous and it didn't need any assistance from the methyl groups alpha to the carbonyl group as suggested in earlier literature.[9c] The appearance of the lactam **14** can be measured spectrophotometrically to ascertain the extent of deprotection.[6] Further work is in progress to utilize **2** as a protective agent in the synthesis of site-specifically modified DNA.

In conclusion we have developed a new protective group for the exocyclic amino groups of deoxy nucleosides. We believe that this protective group will find general use for the protection of the amino functionality wherein the conventional methods are ineffective.

Acknowledgements

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Supplementary data

The supplementary data including ^1H NMR and EI-MS or FAB-MS data for compounds **6**, **11**, **12**, **13** is available online with the paper in Science Direct. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.01.028.

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12. Typical procedure of synthesis of deoxynucleosides **11**, **12** or **13**: To a suspension of the previously dried (over P_2O_5 at 40 °C for 16 h and by coevaporation with pyridine) deoxynucleoside (**8**, **9** or **10**, 2 mmol) in ahydrous pyridine (8 mL), TMS-Cl (6 mmol) was added and the reaction mixture was stirred at 23 °C for 10 min. This was followed by the successive addition of the acid chloride **6** (2.5 mmol) in dichloromethane (8 mL) then 4-dimethlyaminopyridine (1 mmol). After 16 h at 23 °C the reaction mixture was quenched with MeOH (10 equiv), stirred for 15 min and then diluted with water (10 equiv) and stirred for an additional 30 min. After removing the volatiles the residue was dissolved in CH_2Cl_2 (50 mL) and the organic layer was washed with water (2×25 mL), then brine (25 mL) and finally dried (anhyd Na_2SO_4) and evaporated. The residue was subjected to silica gel chromatography to give the pure product, which was triturated with ether/hexane mixture (1:1). The solid obtained was filtered and dried over P_2O_5 at 23 °C for 16 h to obtain the derivatized deoxynucleoside (**11**, **12** or **13**) in good yield (60–65%).
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15. Typical procedure of deprotection of deoxynucleosides **11**, **12** or **13**: The previously dried (over P_2O_5 at 40 °C for 24 h under vacuum) N-acyl nucleoside (**11**, **12** or **13**; 0.2 mmol) was dissolved in anhydrous DMSO (2 mL, stored over 3 Å molecular sieves) and treated with anhydrous TBAF (0.4 mmol, dried at 65 °C under vacuum for 16 h prior to usage) at 50 °C for 1–2 h. TLC (10% MeOH–CH_2Cl_2) analysis of the reaction mixture after this time showed the disappearance of starting N-acyl derivative and complete conversion to natural deoxy nucleosides **8**, **9**, **10** and the lactam **14**. No other products were observed on TLC indicating the quantitative conversion of the reactions.
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