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Polymer supported carbodiimide strategy for the synthesis of *N*-acylated derivatives of deoxy- and ribo purinenucleosides using active esters

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Abstract—A cost-effective synthetic strategy has been used for the selective protection of the exocyclic amino function of purine nucleosides. Instead of using the common protecting groups in their chloride or anhydride forms, the less expensive and nontoxic acidic form was chosen. The acids were first activated to an active ester form using DCC and further successfully used for N-acylation of purine nucleosides. The contamination of the *N*-acylated product with DCU was inconvenient and was avoided by use of polymer supported-carbodiimide that has the additional advantage of reusability.

During the past few years, oligodeoxyribonucleotides (ODNs) have become the focus of active research due to recognition of antisense compounds as potential therapeutic agents.[1–4] One such compound, VitraveneTM, has been approved by the FDA for the treatment of CMV-retinitis. Several other ODNs have demonstrated pre-clinical efficacy[5] and a number of antisense oligonucleotide drugs are undergoing human clinical trials for the treatment of viral infections, cancer,[6] AIDS, and a range of inflammatory disorders.

One of the most important steps in the chemical synthesis of ODNs is selective protection and deprotection of different nucleophilic sites within the monomeric nucleoside building blocks, including protection of exocyclic amino groups of the nucleoside bases. The classical procedure developed by Khorana and co-workers[7] for both deoxy- and ribonucleosides involves peracylation of the nucleosides via acylation of both the hydroxyl groups of sugar and the exocyclic amino group simultaneously, followed by selective hydrolysis of the esters, leaving the *N*-acylated nucleosides. Jones procedure[8] involves prior silylation of hydroxyl functions for transient protection, followed by selective acylation of the amino function. Strategies developed to selectively protect the amino function have led to the discovery of several competent groups[9–17] with a number of groups reported from our laboratory.[18–23]

Conventionally, all reagents that have been reported so far for the protection of exocyclic function of nucleosides are either in chloride or anhydride form. In the present communication, installation of the protecting group has been carried out by an entirely different method. Instead of using acyl halides, the acids have been activated to their corresponding esters using *p*-nitrophenol. The in situ activated esters were used as protecting reagents and coupled with the exocyclic amino function to generate the –CO–NH– bond in the presence of DCC, a well-established strategy in peptide synthesis. While using DCC as a coupling agent, the contamination of the product with dicyclohexylurea (DCU) is very common. Water-soluble EDCI was reported as an alternative reagent, which can be subsequently eliminated by an aqueous extraction. Also, more soluble forms of DCC have been reported,[24] removable with DCM. To overcome the contamination problem, polymer supported-cyclohexylcarbodiimide[25] (PSCC) is successfully used in the present work, which can be easily separated from the reaction mixture through filtration. The advantage

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of using PSCC is that the urea byproduct can be readily converted back to the active carbodiimide and reused.[26] N^6-Bz-2′-dA, N^6-phenoxyacetyl-2′-dA, N^2-*i*-Bu-2′-dG, N^6-Bz-A, N^6-phenoxyacetyl-A, and N^2-*i*-Bz-G have been prepared using this approach.

The strategy presented here eliminates the need for using conventional two-step procedures. The N-acylation achieved is found to be absolutely selective and most importantly, acylation of the sugar hydroxyl group is not observed. The advantages envisaged in this process are significant. One of these happens to be the crystalline nature of the product, that is, *N*-acylated nucleosides, which is a deciding factor while streamlining the purification process, as well as improving yields of the *N*-acylated products. However, contamination of the product with DCU[27] is the outcome undesired of this process. To decrease the DCU content, many molar ratios have been attempted both at the active ester formation step as well as the nucleoside protection step (Table 1). While attempting benzoylation of dA/A, five molar ratios have been tried, 1:1:1, 1:1:1.25, 1:1:1.5, 1:1:1.75, and 1:1:2 (acid:nucleoside:DCC), respectively. While using a 1:1:1 ratio, contamination with DCU was found to be minimal with the yield also being low (50%). A ratio of 1:1.25 was found to be optimal, resulting in 80% yield with a low level of contamination (Table 1). This strategy has been successfully applied to other protecting groups, such as phenoxyacetyl and isobutyryl (data not shown), and the products were found to be in the same ratio. Using this strategy, *N*-protected derivatives of 2′-dG and 2′-dA using three new protecting groups have been synthesized and are reported elsewhere.[28]

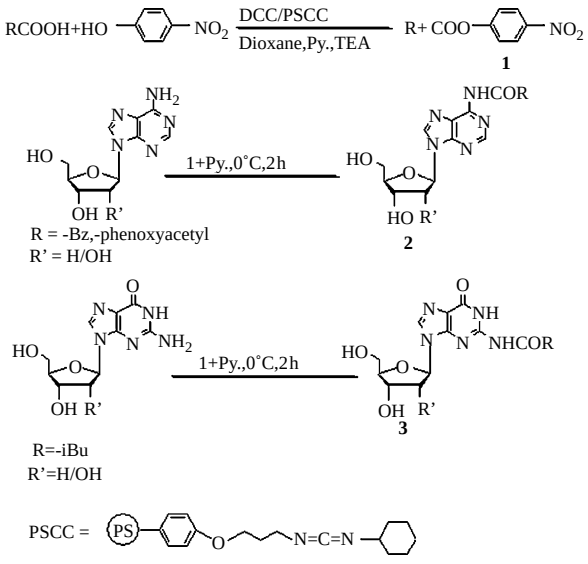
In the quest for an alternative to DCC, polymer-bound carbodiimide[25] was used successfully, yielding a very promising result with an enhanced yield, as well as being a simplified purification process. Since PS-carbodiimide is easy to separate from the reaction mixture, a facile method has been successfully developed. The used polymer-supported reagent can further be recycled (PSCC–PSCU–PSCC), as previously mentioned. PSCC makes this strategy further versatile, as well as cost-effective. The regenerated PSCC was found to be stable at room temperature and useful for another round of N-acylation. We have used regenerated PSCC four consecutive times and the yield of the protected derivatives was found to be comparable, further confirming the reusability of PSCC. With the potential for antisense technology in the treatment of inflammation, viral diseases, and cancers,[29] the anticipated demand for modified ODNs may be on the metric ton scale in the near future and we envisaged the present method to effectively facilitate cost reduction in the synthesis of ODNs.

Table 1. Synthesis of N^6-Benzoyl-dA using different molar ratios of acid, nucleoside, and DCC

Molar ratio acid: nucleoside:DCC	Yield (%)	FAB-MS of benzoic *p*-nitrophenyl ester	FAB-MS of N^6-benzoyl-dA
1:1:1	50	242	351
1:1:1.25	80	242	350
1:1:1.5	82	242	350
1:1:1.75	86	241.8	351
1:1:2	94	242	348

The PSCC was purchased from Argonaut Technologies, San Carlos, CA. ^{1}H NMR spectra were recorded on Bruker DRX 300. Small amounts of all the prepared *N*-acylated derivatives were hydrolyzed to get back the starting nucleosides for confirmation of their structures. Percentage purification of the products was checked on HPLC (Pharmacia LKB-DBF) using reverse-phase columns. *N*-Acylated nucleosides were purified using a Whatman RP-C_{18} column using 20 mM ammonium acetate buffer (pH 3.5) as buffer A and 20 mM ammonium acetate buffer (pH 3.5)/methanol (90:10 v/v) as buffer B at a flow rate of 0.75 mL/min, with detection at 260 nm and column temperature set at 30 °C. Buffer B (30%) was passed for 5 min and was increased as a linear gradient for 10 min. Buffer B (100%) was run for another 40 min. The mass characterization was done on a Jeol-SX 102/DA-6000 spectrometer using argon as the FAB gas (6 kV, 10 mA).

General method for N-acylation of nucleosides using DCC (Scheme 1). Recrystallized and dried benzoic acid/phenoxyacetic acid (1 mmol) was dissolved in anhyd 1,4-dioxane (5 mL) under an atmosphere of nitrogen. A solution of *p*-nitrophenol (1.2 mmol) in anhyd 1,4-dioxane (5 mL) was added to it. Protection from moisture was essential. The reaction mixture was made basic by addition of pyridine (0.5 mL) and TEA (0.5 mL). After 10 min of stirring, DCC (1.25 mmol) was added to it [DCC was melted in a sealed tube after weighing (mp 35 °C) and then mixed to the r.m.]. The reacting mixture was allowed to stir at room temperature for 2 h. Completion of the reaction was assessed by the absence of the starting material on TLC. The reaction mixture was cooled to 0 °C and anhyd 2′-dA/A (1 mmol) suspended in 5 mL of pyridine was added to the activated ester (**1**) with the help of a syringe gradually over a



Scheme 1.

period of 15 min. After 10 min, a second batch of DCC (1.25 mmol) was added again and the r.m. was allowed to stir for a further 2 h. The reaction mixture was filtered under vacuum to remove the precipitated DCU and the filtrate was evaporated to a gum under vacuum. It was then dissolved in 5% aqueous $NaHCO_3$ solution and extracted with DCM (4× 5 mL). The organic layer was washed with water to remove released *p*-nitrophenol until the water became colorless, dried over Na_2SO_4, and filtered and evaporated to dryness. The residue was extracted in diethyl ether and crystallized further to furnish the product (**2**). The above-described method was repeated by substituting isobutyric acid in place of benzoic/phenoxyacetic acid with 2′-dG/G to get the *N*-acylated derivatives (**3**). The ^1H NMR data were found to be comparable to the earlier reported literature.[8] The FAB-MS for N^6-phenoxyacetyl-dA/A and N^2-isobutyryl-dG/G were found to be comparable to the authentic samples (data not shown).

General method for N-acylation using PS-carbodiimide. The respective acids (1 mmol) were dissolved in 5 mL of dry 1,4-dioxane and PS-carbodiimide (2.5 mmol) was added. The reaction mixture was made basic by addition of pyridine (0.5 mL) and TEA (0.5 mL). With stirring a solution of *p*-nitrophenol (1.2 mmol) dissolved in 5 mL of anhyd 1,4-dioxane was added to it through a syringe in over 0.5 h. The reaction mixture was allowed to stir at room temperature for 2 h and was then transferred to an ice bath. Solutions of the respective 2′-deoxyribonucleosides or ribonucleosides (1 mmol) in 5 mL of pyridine were added with a syringe. After 2 h of stirring, the reaction was checked for completion by TLC. Absence of starting material confirmed completion of the reaction and the reaction mixture was filtered to remove PSCU and then evaporated to a gum, dissolved in 5% aqueous $NaHCO_3$ solution, and extracted with DCM (4× 5 mL). The organic layer was washed with water, dried over Na_2SO_4, and filtered. Combined DCM solutions were concentrated and then crystallized in diethyl ether.

Conditions for deprotection. The removal conditions for all the three protecting groups were studied by treating the *N*-protected nucleosides with 40% ammonia at 25, 40, and 50 °C. Reactions were quenched after 0.5, 1, 2, 3, 4, 5, and 6 h duration. After hydrolysis, the mixtures were analyzed for respective deprotected nucleosides on semi-preparative TLC and subsequent estimation by UV. The results were then matched with the same procedure carried out by HPLC. Results were found to be comparable to those of an authentic sample of *N*-protected nucleosides (data not shown).

Procedure for regeneration of PS-cyclohexylcarbodiimide (PSCC). The polymer bound cyclohexylurea (PSCU; 1 g of 1.25 mmol/g) recovered after N-acylation was suspended in DCM (20 mL) in a three-neck round-bottomed flask (100 mL) equipped with a silicon rubber septum and a reverse filter funnel under exclusion of moisture and air.[26] Phosphorus oxychloride ($POCl_3$) (0.38 g, 2.5 mmol) was added slowly over a period of 10 min to the above suspension. The mixture was vortexed gently for 4–6 h and the supernatant was removed by reverse filtration and replaced by 10% aqueous NaOH (5 mL) and fresh dichloromethane (20 mL). The suspension was shaken further for 1 h and the aqueous supernatant was removed. The polymer was washed with THF (2× 25 mL) to remove excess water and dried under vacuum to furnish PSCC.

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References and notes

1. Crooke, S. T. *Annu. Rev. Med.* **2004**, *55*, 61.
2. Kurreck, J. *Eur. J. Biochem.* **2003**, *270*, 1628.
3. Goodchild, J. *Curr. Opin. Mol. Ther.* **2004**, *6*, 120.
4. Sanghvi, Y. S. In *Comprehensive Natural Products Chemistry*, Barton, D. H. R., Nakanishi, K. Eds.; *DNA and Aspects of Molecular Biology*, Vol. 7; Kool, E. T.; Ed.; Pergamon Press: Elmsford, NY, 1999; p 285.
5. Winkler, K. E. *Nature* **2004**, *3*, 823.
6. (a) Pirollo, K. F.; Rait, A.; Sleer, L. S.; Chang, E. H. *Pharmacol. Ther.* **2003**, *99*, 55; (b) Prasad, G.; Wang, H.; Hill, D. L.; Zhang, R. *Curr. Opin. Mol. Ther.* **2004**, *4*, 347.
7. Schaller, H.; Weiman, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821.
8. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.
9. Jones, R. A.; Fritz, H. J.; Khorana, H. G. *Biochemistry* **1987**, *17*, 1268.
10. Barnett, R. W.; Letsinger, R. L. *Tetrahedron Lett.* **1981**, *22*, 991.
11. Buchi, H.; Khorana, H. G. *J. Mol. Biol.* **1972**, *72*, 251.
12. Furusawa, K.; Sakai, T.; Tsuda, K.; Shimura, Y. *Nucleic Acids Symp. Ser.* **1982**, *12*, 21.
13. Froehler, B. C.; Matteucci, M. D. *Nucleic Acids Res.* **1983**, *11*, 8031.
14. Ogilvie, K. K.; Nemer, M. J.; Hakimelahi, G. H.; Proba, Z. A.; Lucas, M. *Tetrahedron Lett.* **1982**, *23*, 2615.
15. Heikkila, J.; Balgobin, N.; Chattopadhyaya, J. *Acta Chem. Scand., Ser. B* **1983**, *37*, 263.
16. Hayakawa, Y.; Kato, N.; Uchiyama, M.; Kajino, Y.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2400.
17. Kume, A.; Sekine, M.; Hata, T. *Chem. Lett.* **1983**, 1597.
18. Mishra, R. K.; Misra, K. *Nucleic Acids Res.* **1986**, *14*, 6197.
19. Singh, R. K.; Misra, K. *Indian J. Chem. Sec. B* **1988**, *27*, 409.
20. Dikshit, A.; Chaddha, M.; Singh, R. K.; Misra, K. *Can. J. Chem.* **1988**, *66*, 2989.
21. Nagaich, A. K.; Misra, K. *Nucleic Acids Res.* **1989**, *17*, 5125.
22. Singh, R. K.; Dikshit, A.; Chaddha, M.; Watal, G.; Misra, K. *J. Sci. Ind. Res.* **1990**, *49*, 441.
23. Misra, A.; Tripathi, S.; Misra, K. *Indian J. Chem. Sec. B* **2002**, *41*, 1454.
24. Gesquiere, J.-C.; Tartar, A. *J. Org. Chem.* **1979**, *44*, 5000.
25. Keck, G. E.; Sanchez, R.; Wager, C. A. *Tetrahedron Lett.* **2000**, *41*, 8673.
26. Fujibayashi, R. (Sumikin Kakoh Co. Ltd.), Jpn. Pat. Appl. No. 95-37375, February 24, 1995.

27. Anderson, N. G. *Practical Process Research and Development*; Academic Press: New York, 2000, p 58.
28. Tripathi, S.; Misra, K.; Sanghvi, Y. S. *Synth. Commun*. **2005**, *37*, in press.
29. Sanghvi, Y. S.; Andrade, M.; Deshmukh, R. R.; Holmsberg, L.; Scozzari, A. N.; Cole, D. L. In *Manual of Antisense Methodology*; Hartmann, G., Endres, S., Eds.; Kluwer Academic: Norwell, MA, 2000.