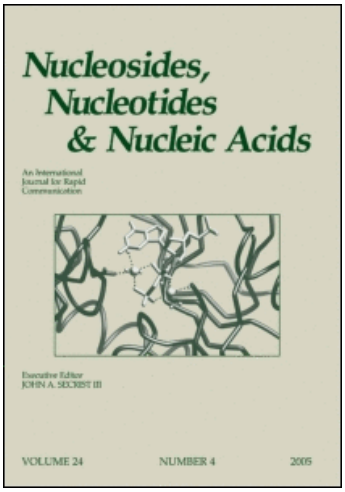


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Large-Scale Synthesis of "Cpep" RNA Monomers and Their Application in Automated RNA Synthesis

R. T. Pon[a]; S. Yu[a]; T. Prabhavalkar[b]; T. Mishra[b]; B. Kulkarni[b]; Y. S. Sanghvi[c]
[a] Department of Biochemical and Molecular Biology, University of Calgary, Calgary, Canada [b] Innovassynth Technologies (India) Ltd., Khopoli, India [c] Rasayan Inc., Encinitas, California, USA

To cite this Article Pon, R. T. , Yu, S. , Prabhavalkar, T. , Mishra, T. , Kulkarni, B. and Sanghvi, Y. S.(2005) 'Large-Scale Synthesis of "Cpep" RNA Monomers and Their Application in Automated RNA Synthesis', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 777 — 781
To link to this Article: DOI: 10.1081/NCN-200060150
URL: http://dx.doi.org/10.1081/NCN-200060150

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):777–781, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200060150

Taylor & Francis
Taylor & Francis Group

LARGE-SCALE SYNTHESIS OF ''Cpep'' RNA MONOMERS AND THEIR APPLICATION IN AUTOMATED RNA SYNTHESIS

R. T. Pon and S. Yu ▫ *Department of Biochemical and Molecular Biology, University of Calgary, Calgary, Alberta, Canada*

T. Prabhavalkar, T. Mishra, and B. Kulkarni ▫ *Innovassynth Technologies (India) Ltd., Khopoli, India*

Y. S. Sanghvi ▫ *Rasayan Inc., Encinitas, California, USA*

▫ *Small interfering RNAs (siRNA) are the latest candidates for oligonucleotide-based therapeutics. Should siRNA be successful in clinical trials, a huge demand for synthetic RNA is anticipated. We believe that 1-(4-chlorophenyl)-4-ethoxypiperidin-4-yl (Cpep) is an ideal 2′-protecting group for large-scale syntheses. Unlike 2′-silyl groups, mild acid hydrolysis instead of fluoride ion is used for the 2′-deprotection. The syntheses of 2′-Cpep protected nucleosides (A, C, G, and U) has been accomplished on a 0.5 Kg scale. The 2′-Cpep monomers were transformed into 3′-O-phosphoramidites for conventional automated solid-phase synthesis. Cost-effective processes for large-scale synthesis of Cpep monomers and initial automated solid-phase synthesis are demonstrated.*

Keywords 2′-Protecting Groups, Phosphoramidites, Oligoribonucleotides

INTRODUCTION

Oligonucleotides that modulate gene expression are recognized as powerful research tools and potential therapeutic agents. The antisense oligonucleotides are the most advanced, with one product on the market, but other oligonucleotides technologies, such as ribozymes, immunostimulatory CpG sequences, aptamers, miRNA and RNAi are emerging rapidly. With the advent of these products in the clinic and market, there will be a huge manufacturing demand. While the synthesis of DNA has become routine and manufacturing costs have dropped significantly, the synthesis of RNA remains a challenge. Herein, we present our efforts toward large-scale synthesis of 2′-Cpep RNA monomers as the first step in our program to reduce large-scale RNA production costs.

Address correspondence to R. T. Pon, Department of Biochemical and Molecular Biology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The synthesis of RNA using solid-phase methods on small-scales ($\leq$1 μmol) has been routine for a number of years. Three 2′-protecting groups: t-butyldimethylsilyl[1] (TBDMS); [(triisopropylsilyl)oxy]methyl[2] (TOM); and bis(2-acetoxyethoxy)-methyl[3] (ACE) have been successfully commercialized for the synthesis of RNA, both in large number and large scale. Among these groups, the 2′-TBDMS[1] chemistry is the oldest and most commonly used, but it has not provided the quality and yield of oligonucleotide possible in DNA synthesis. As a result, in 1998, 2′-TOM[2] and 2′-ACE[3] groups were introduced as more satisfactory alternatives. Although oligoribonucleotide synthesis with either 2′-TOM and 2′-ACE chemistry is commercially available, the monomeric reagents are not accessible at reasonable cost, especially in large quantities.

We believe that low-cost RNA monomers will be crucial to the success of RNA based therapeutics. In this regard, we believe that the 2′-Cpep RNA monomers reported by Reese[4] may ultimately provide cost and chemistry advantages currently unattainable with 2′-TOM or 2′-ACE groups. The Cpep reagent is crystalline, easy to prepare in large scales, and it has unique hydrolysis properties, which avoid problems with intemucleotide linkages migration during acidic 2′-deprotection.

RESULTS AND DISCUSSION

Synthesis of Cpep Reagent

The Friedel-Crafts acylation of ethylene with 3-chloropropionyl chloride **1** in DCM furnished excellent yield of 1,5-dichloropentan-3-one[5] **2** (Figure 1). Next,

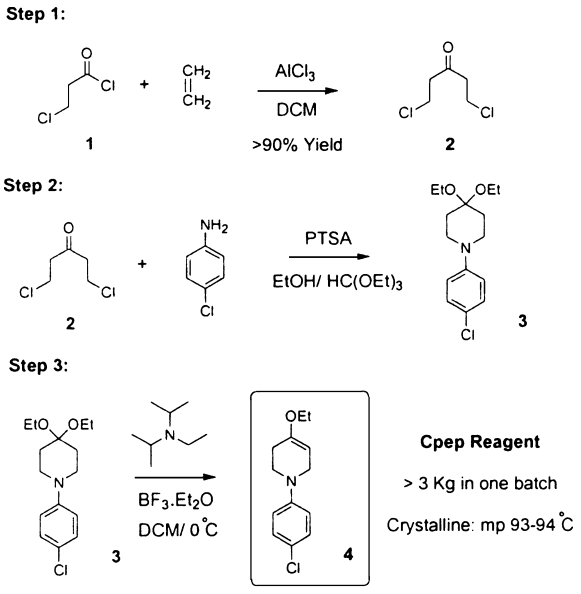


FIGURE 1 Cpep synthesis on Kg scale.

crude **2** was refluxed with 4-chloroaniline in presence of PTSA followed by addition of triethyl orthoformate. Upon completion of the reaction, 4,4-diethoxypiperdine analog **3** was isolated as a crystalline product in >90% yield. Reaction of Hünigs base and $BF_3 \cdot Et_2O$ in DCM at 0°C furnished the desired Cpep reagent **4** in >90% yield as a crystalline product.

Synthesis of Cpep-Protected Nucleoside 9

Appropriately base protected ribonucleosides were treated with 1.2 equivalents of triisopropylsilyl chloride (TIPSCI) to furnish **6** in >80% yield. The isolation of 6 was significantly simplified by using a mixture of ACN/pyridine during 3′- to 5′-protectiOn. The reaction of Cpep reagent **4** with **6** in the presence of DCA provided **7** in >70% yield. Hygroscopic PTSA was replaced with DCA as a more easily manageable acid during scale-up studies. Deprotection of TIPS from **7** was carried out with TEAF in THF to furnish **8** in >75% yield. TBAF, traditionally used, was replaced with TEAF as a cheaper and easily separable deblocking reagent in the scale-up process. Lastly, treatment of **8** with 1.1 eq. of DMT-C1 in pyridine provided **9** as an amorphous powder after column chromatography. This series of reactions were carried out on >200 g scale to establish the scale-up route (Figure 2).

Phosphitylation Reaction of 9

Phosphitylation of **9** with 2-cyanoethyl diisopropyl-chlorophosphoramidite resulted in faster reaction, however with formation of byproducts. Use of 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (Phos) instead furnished a

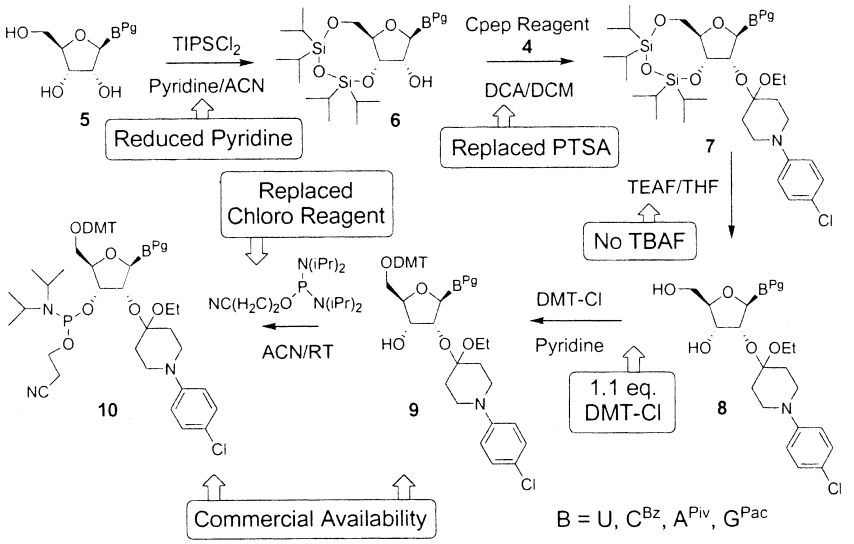


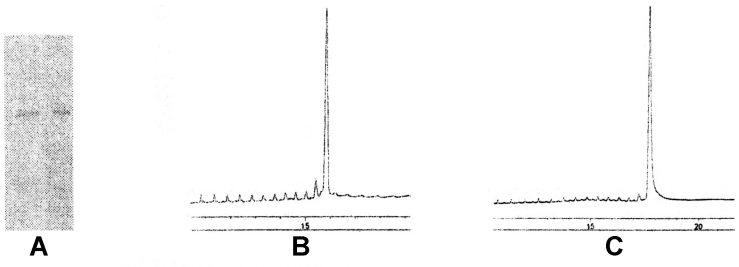
FIGURE 2 Synthesis of 2′-O-Cpep phosphoramidites.

much cleaner reaction with no byproducts formed. Due to its inherent safety, the Phos reagent seems to be the right choice for phosphitylation on large scales. The purity of phosphoramidites **10** prepared from Pharma grade Phos reagent (Digital Specialty Chemicals, Canada) was >98% (^{31}P NMR).

Oligonucleotide Synthesis

A test sequence rU20 was synthesized on CPG solid-support (1 μmol) using an ABI 394 DNA synthesizer. A 0.1 M solution of rU phosphoramidite **10** was employed with 0.25 M ethylthiotetrazole (ETT) solution as activator and a 10 min coupling time. Standard RNA synthesis cycles were utilized for deprotection (3% DCA in DCM), capping (Ac_2O/2,6-lutidine/THF) and oxidation (I_2/H_2O/pyridine/THF). The oligonucleotide was cleaved from the solid-support and base deprotection was done by treatment with 3:1 NH_4OH/EtOH (RT, 48 h). The deprotection of the 2′-Cpep groups was accomplished in 4:6 formate buffer (0.5 M, pH 2.5)/dimethylacetamide (40°C, 5 h). CGE analysis showed >55% product (Figure 3). This was slightly less than using 2′-TBDMS but protocol optimization has not been completed.

The synthesis and scale-up of the four Cpep monomers **9** (B = U, C^{Bz}, A^{piv} and G^{pac}) has been accomplished on a large scale. During scale-up, several process improvements have been made to yield a safe, environmentally clean, robust and economical synthesis of these new RNA monomers. Presently, the cost and accessibility of 2′-ACE and 2′-TOM RNA monomers are limitations to large-scale drug production. We have shown that 2′-Cpep monomers can be prepared in large scale at low cost. The synthesis of mixed RNA sequences on a large scale is currently under investigation and the results will be published in due course.



A. 24% Polyacrylamide/7M urea gel of crude U20 made with 2'-Cpep (left) and 2'-TBDMS (right)

B. Capillary gel electrophoresis of crude U20 made with 2'-Cpep

C. Capillary gel electrophoresis of crude U20 made with 2'-TBDMS

FIGURE 3 Analysis of 2′-Cpep and TBDMS rU 20-mers.

REFERENCES

1. Ogilvie, K.K.; Damha, M.J.; Usman, N.; Pon, R.T. Developments in the chemical synthesis of naturally occurring DNA and RNA sequences with normal and unusual linkages. Pure Appl. Chem. **1987**, *59*, 325–330.
2. Pitsch, S.; Weiss, P.A.; Jenny, L.; Stutz, A.; Wu, X. Reliable chemical synthesis of oligoribonucleotides (RNA) with 2′-*O*-[(ttriisopropylsilyl)oxy]methyl (2′-*O*-tom)-protected phosphoramidites. Helv. Chim. Acta **2001**, *84*, 3773–3795.
3. Scaringe, S.A. RNA oligonucleotide synthesis via 5′-silyl-2′-orthoester chemistry. Methods **2001**, *23*, 206–217.
4. Lloyd, W.; Reese, C.B.; Song, Q.; Vandersteen, A.; Vistintin, C.; Zhang, P.-Z. Some observations relating to the use of 1-aryl-4-alkoxypperidin-4-yl groups for the protection of the 2′-hydroxy functions in the chemical synthesis of oligoribonucleotides. J. Chem. Soc., Perkin Trans. 1 **2000**, 165–176.
5. Owen, G.R.; Reese, C.B. A convenient preparation of tetrahydro-4*H*-pyran-4-one. J. Chem. Soc., C **1970**, 2401–2403.