

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

### SYNTHESIS AND EVALUATION OF PROLYL CARBAMATE NUCLEIC ACIDS (PrCNA)

Meena<sup>a</sup>; V. A. Kumar<sup>a</sup>; K. N. Ganesh<sup>a</sup>

<sup>a</sup> Division of Organic Chemistry (Synthesis), National Chemical Laboratory, Pune, India

Online publication date: 31 March 2001

**To cite this Article** Meena, Kumar, V. A. and Ganesh, K. N.(2001) 'SYNTHESIS AND EVALUATION OF PROLYL CARBAMATE NUCLEIC ACIDS (PrCNA)', *Nucleosides, Nucleotides and Nucleic Acids*, 20: 4, 1193 — 1196

**To link to this Article:** DOI: 10.1081/NCN-100002517

**URL:** <http://dx.doi.org/10.1081/NCN-100002517>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## SYNTHESIS AND EVALUATION OF PROLYL CARBAMATE NUCLEIC ACIDS (PrCNA)

Meena, V. A. Kumar, and K. N. Ganesh\*

Division of Organic Chemistry (Synthesis), National Chemical Laboratory, Pune 411008, India

### ABSTRACT

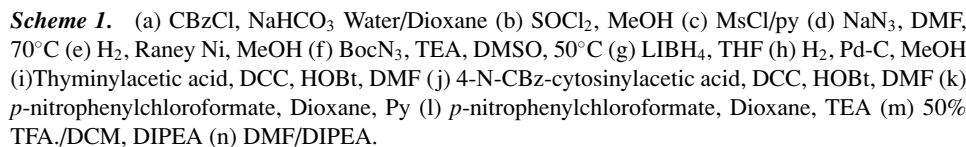
Carbamate linked prolyl nucleic acids are obtained in high yield and purity under mild conditions in solution and solid phase. *p*-Nitrophenylchloroformate is used as the activating reagent for alcohol. Homooligomers of PrCNA do not bind to DNA. The introduction of this modification in PNA sequences destabilizes the triplexes, inspite of enhancement in the base stacking.

Carbamate linked nucleic acids are known to bind with complementary DNA strands with higher thermal stability (1,2). Carbamate linkage being uncharged these are expected to be capable of penetrating the cell membrane and are more stable towards enzymes (3). Earlier, prolyl nucleic acids having peptide backbone were prepared in our laboratory and it was found that the homooligomers of prolyl PNA did not bind to complementary DNA strand (4). This was probably because of the constraint in the flexible PNA backbone (5). We replace the peptide linkages in prolyl nucleic acids with carbamate linkages thus increasing the number of atoms in the backbone while keeping the stereogenic centers intact. Here, we report the synthesis and the binding studies of the carbamate linked prolyl nucleic acids.

The activated monomer building blocks **4a** and **4b** were synthesized from *trans*-L-hydroxy proline **1** (Scheme 1). After stepwise protection of ring nitrogen as benzylcarbamate and acid function as methyl ester the hydroxy group was converted to the corresponding azide in two steps. The azide was then selectively hydrogenated using Raney Ni and the resultant amine was protected as *t*-butoxycarbamate. The

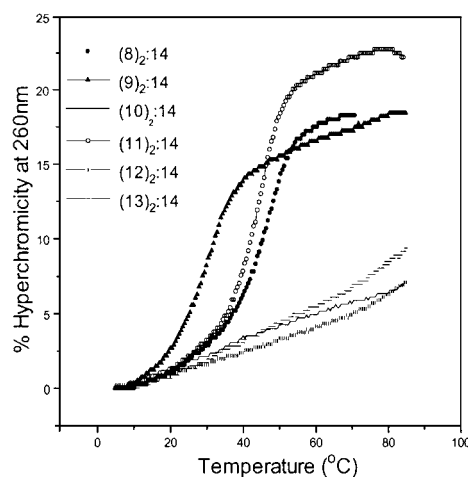
---

\*Corresponding author.



The activated monomer synthons **4a** and **4b** along with standard PNA monomers (**8**) were used to synthesize the oligomer sequences **6** (in solution phase) (**1,2**) and **7–13** (on solid support) using standard protocols. The sequences **7–13** were cleaved from the support using standard conditions used for the cleavage of peptides (**8**). Stability of the carbamate linkage under these conditions was established by independent treatment of **6** with TFMSA/TFA and methanolic ammonia and HPLC and mass spectral analysis of the samples. The carbamate linkage was found to be stable in acidic condition but degradation was observed under basic condition. The oligomers **7–13** were purified by FPLC and purity was checked by HPLC. The homogeneity of each of the oligomer was established by MALDI-TOF

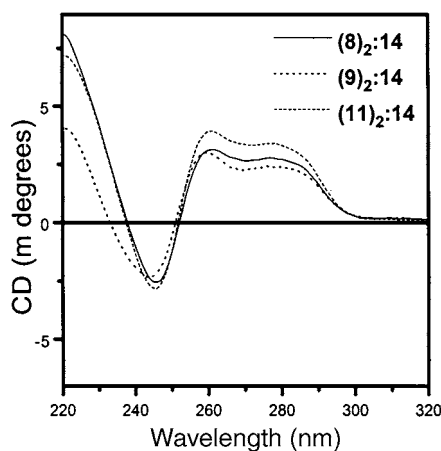
**Scheme 2.**



**Figure 1.** Melting curves for (PrCNA/PNA)<sub>2</sub>: DNA complexes in Sodium phosphate buffer at pH 7.3. Concentration of DNA strand was taken as 1.5  $\mu$ M.

mass spectrometry (7). The effect of the carbamate linked prolyl units on the structural features of the backbone of the oligomers was studied using CD spectroscopy. Compounds **5**, **6** and **7** showed sequential enhancement in the CD signal at 260 nm with increasing number of three prolyl thymine units and plateaued in the prolyl octamer.

The UV melting studies of the complexes of oligomers sequences **8–13** with complementary DNA strands **14–15** were performed to evaluate the thermal stability (Fig. 1). The homooligomers **12** and **13** did not bind to DNA. PNA sequence, **10**, with T\* at the center of the sequence did not bind to DNA. When the modification is at the N-terminus as in PNA **11**, marginal destabilization compared to PNA<sub>2</sub>:DNA



**Figure 2.** CD curves for triplexes in Sodium phosphate buffer at pH 7.3.

**Table 1.** UV Melting Temperatures of Triplexes

Complexes	(8) <sub>2</sub> :14	(9) <sub>2</sub> :14	(10) <sub>2</sub> :14	(11) <sub>2</sub> :14	(12) <sub>2</sub> :14	(13) <sub>2</sub> :15
T <sub>m</sub> °C	44.5	31.5	—	44	—	—
% Hyperchromicity	18.2	18.4	6.8	22.2	7.1	9.3

complex was observed whereas the C-terminal modification in **9** destabilized the triplex by 13°C. The reassociation process of DNA hybridization was monitored by decrease in absorbance with lowering temperature. Absorbance Vs temperature profiles for PrCNA:DNA complexes showed that the rate of reassociation to form triplexes is slower than the rate of dissociation and is similar to DNA:PNA complexes. CD curves obtained for complexes (9)<sub>2</sub>:14 and (11)<sub>2</sub>:14 were similar in pattern to (8)<sub>2</sub>:14 (Fig. 2).

In conclusion, we have successfully developed solid phase synthesis chemistry for carbamate linked oligomers and the insertion of prolyl carbamate linkage into PNA. The results from DNA binding studies show that carbamate linked prolyl homooligomers do not bind to DNA possibly due to incompatible internucleobase distances in the PrCNA. Placement of carbamate linkage either at C-terminal or N-terminal in PNA causes destabilization of triplexes.

## REFERENCES

1. Stirchak, E. P.; Summerton, J. E.; Weller, D. D. *J. Org. Chem.* **1987**, *52*, 4202.
2. Stirchak, E. P.; Summerton, J. E.; Weller, D. D. *Nucl. Acids Res.* **1989**, *15*, 6129.
3. Habus, I.; Tesamani, J.; Agarwal, S. *BioMed. Chem. Lett.* **1994**, *4*, 1065.
4. Gangamani, B. P.; Kumar, V. A.; Ganesh, K. N. *Tetrahedron.* **1999**, *55*, 177.
5. Ganesh, K. N.; Nielsen, P. E. *Curr. Org. Chem.* **2000**, *9*, 916.
6. Letsinger, R. L.; Ogilvie, K. K. *J. Org. Chem.* **1967**, *32*, 296.
7. Compound **4a** <sup>1</sup>H NMR in CDCl<sub>3</sub> δ ppm 8.25 (d 2H phenyl J = 0.2), 7.4 (d 2H phenyl J = 0.2), 7 (s 1H thy 6H), 4.5 (m 5H OMe & Pro C5), 1.9 (3H Thy Me), 1.4 (d 9H Boc). Compound **4b** <sup>1</sup>H NMR in CDCl<sub>3</sub> δ ppm 8.25 (d 2H nitroPhe), 7.6 (d 1H Cyt C6 J = 0.15), 7.25 (m 7H cbz, nitroPhe, Cyt C5), 5.2 (s 2H benzyl CH2), 4.5 (m 5H benzyl CH2, Proline C2-methyl, C5), 1.4 (s 9H t-Boc). Mass (FAB) m/z = 667(M+1), 689(M+Na) calculated mass 666. Compound **6** Mass(FAB) m/z = 691 (M+1), 713(M+Na) Calculated mass 690. Compound **9** Mass (MALDI TOF) m/z = 2268.8 calculated mass 2259 and **10** m/z = 2266.5 calculated mass 2259.
8. Nielsen, P. E.; Egholm, M. Eds. In *Peptide Nucleic Acids: Protocols and Applications*, 1999, Horizon Scientific Press, Norfolk, U.K.



## **Request Permission or Order Reprints Instantly!**

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the [U.S. Copyright Office](#) for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on [Fair Use in the Classroom](#).

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our [Website User Agreement](#) for more details.

**[Order now!](#)**

Reprints of this article can also be ordered at

<http://www.dekker.com/servlet/product/DOI/101081NCN100002517>