

## Synthesis of an Enantiomerically Pure Carbocyclic DNA Abasic Site Analogue

Martial Thomas , Bertrand Castaing , Jean-Louis Fourrey & Charles Zelwer

**To cite this article:** Martial Thomas , Bertrand Castaing , Jean-Louis Fourrey & Charles Zelwer (1999) Synthesis of an Enantiomerically Pure Carbocyclic DNA Abasic Site Analogue, *Nucleosides and Nucleotides*, 18:2, 239-243, DOI: [10.1080/15257779908043071](https://doi.org/10.1080/15257779908043071)

**To link to this article:** <http://dx.doi.org/10.1080/15257779908043071>



Published online: 04 Oct 2006.



Submit your article to this journal [↗](#)



Article views: 16



View related articles [↗](#)



Citing articles: 3 View citing articles [↗](#)

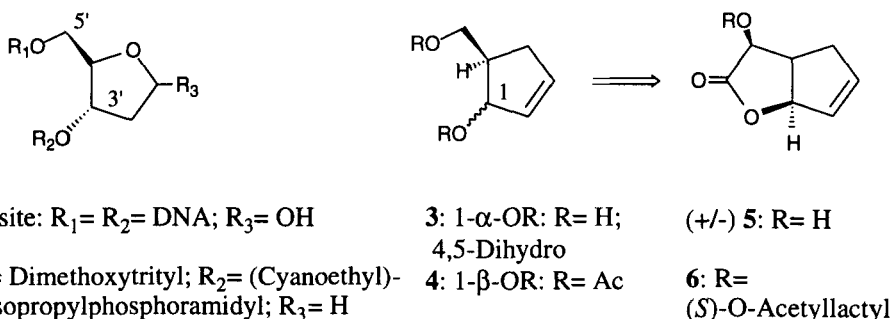
## SYNTHESIS OF AN ENANTIOMERICALLY PURE CARBOCYCLIC DNA ABASIC SITE ANALOGUE

Martial Thomas<sup>a</sup>, Bertrand Castaing<sup>b</sup>, Jean-Louis Fourrey<sup>a\*</sup> and Charles Zelwer<sup>b</sup>

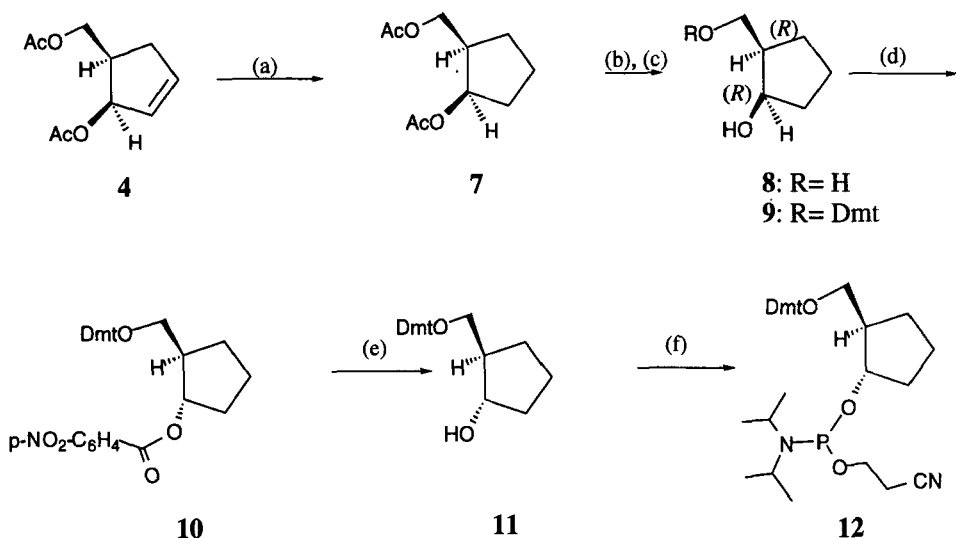
<sup>a</sup> Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, France  
and <sup>b</sup> Centre de Biophysique Moléculaire, CNRS-Université d'Orléans, Rue Charles Sadron, 45071 Orléans Cedex 2, France.

**Abstract:** A short synthetic route to an appropriately derivatized carbocyclic analogue of abasic site residues of DNA is proposed.

Base excision repair mechanisms, which remove purine and pyrimidine damages from genomic DNA, represent essential biological processes which are currently receiving a widespread interest. In general, the preliminary step of such DNA repair is achieved by a glycosylase whose role is to excise altered nucleic acid bases with the formation of an abasic site 1 (AP) (Figure).<sup>1</sup> AP sites are also generated through spontaneous non enzymatic glycosidic bond hydrolysis.



Figure



Dmt= dimethoxytrityl

Reagents: (a)  $\text{H}_2$ , Pd/C, EtOH; (b) MeOH, aq. NaOH (9:1); (c) DmtCl, DBU,  $\text{CH}_2\text{Cl}_2$  (d)  $\text{p-NO}_2\text{C}_6\text{H}_4\text{CO}_2\text{H}$ ,  $\text{PPh}_3$ , DEAD, THF; (e) aq. NaOH, THF; (f) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylethylamine,  $\text{CH}_2\text{Cl}_2$

### Scheme

Whatever their formation pathways, abasic sites consist of a 2'-deoxyribose moiety linked to its nucleoside neighbours through 3'- and 5'-phosphodiester bonding. It is well known that such bonds are prone to undergo  $\beta$ -elimination reactions. Accordingly, the formation of an AP site within a DNA strand generally precedes its scission. This process can be achieved by AP endonucleases or DNA glycosylases which, in some cases, are endowed with an AP lyase activity and cleave the DNA phosphodiester backbone at the 3'-side of the AP site.<sup>1</sup> To study in more details the various aspects of these enzyme activities, there is a need to develop oligonucleotide substrates containing abasic sites analogues which might mimic deoxyribose residues within single strand DNA and exhibit a high affinity for the enzymes.<sup>2</sup>

Indeed, with the advent of efficient synthetic methods to obtain DNA fragments, a number of efforts have been done to design such synthetic analogues to be incorporated in oligodeoxynucleotides. This is the case of 3-hydroxy-(2-hydroxymethyl)-tetrahydrofuran,<sup>2</sup> a very close AP site mimic, whose appropriate phosphoramidite derivative **2** is now commercially available for its incorporation into synthetic

oligonucleotides. Along this research line, to the best of our knowledge, the corresponding carbocyclic analogue **3** of tetrahydrofuran has never been described. Herein, we propose an asymmetric access to **11**, a derivative of (1*S*, 2*R*)-2-(hydroxymethyl)-cyclopentanol **3**<sup>3</sup> and its transformation into phosphoramidite **12**, applicable for the selective incorporation of **3** at any position within an oligonucleotide.

Our synthetic scheme is based on a new application of the optically pure diacetate **4** which can be readily obtained<sup>4</sup> by a simple and short synthetic sequence starting from lactone (+/-) **5** via its optically active derivative **6** (Scheme).<sup>5</sup> Thus, as outlined in the Scheme, compound **4** was hydrogenated in the presence of Pd/C catalyst to give the saturated derivative **7** whose spectral data were in accordance with those previously reported in the achiral series.<sup>6</sup> Compound **7** was deacetylated by an overnight treatment in a methanolic solution containing aqueous sodium hydroxide. Dimethoxytritylation of the resulting diol **8** provided the protected alcohol **9**. Inversion of the configuration of the secondary alcohol function was accomplished using standard Mitsunobu conditions in the presence of *p*-nitrobenzoic acid to obtain compound **10**. Saponification of ester **10** was performed by a sodium hydroxide treatment which gave alcohol **11**. Its corresponding phosphoramidite **12** was prepared by application of standard reaction conditions.

This synthon can now be used for the incorporation of this carbocyclic abasic site analogue into oligonucleotides.

## EXPERIMENTAL SECTION

(1*R*,2*R*) 2-(Acetoxymethyl)-cyclopentanyl acetate **7**: To a solution of diacetate **4** (1 g, 5 mmol) in ethanol (100 ml) was added 100 mg of Pd/C. This mixture was hydrogenated overnight under 50 psi at room temperature to give 901 mg of 2-(acetoxymethyl)-cyclopentanyl acetate **7** as an oil (90%). <sup>1</sup>H nmr (CDCl<sub>3</sub>) δ (ppm): 5.25 (br. t, 1H, H-1); 4.11 (m, 2H, AcOCH<sub>2</sub>); 2.27 (m, 1H, H-2); 2.05 (3H, s, CH<sub>3</sub>CO); 2.03 (3H, s, CH<sub>3</sub>CO); 1.97-1.64 (6H, m, 3xCH<sub>2</sub>). <sup>13</sup>C nmr (CDCl<sub>3</sub>) δ (ppm): 170.7; 170.3; 75.7; 63.3; 42.6; 32.3; 26.5; 21.7; 20.8; 20.6. MS CI *m/z* 201 (MH<sup>+</sup>). Analysis: Calc. for C<sub>10</sub>H<sub>16</sub>O<sub>4</sub>: C 59.98, H 8.06; Found: C 60.12, H 8.27.

(1*R*,2*R*) 2-(4,4-Dimethoxytrityloxymethyl)-cyclopentanol **9**: A solution of diacetate **7** (802 mg, 4 mmol) in 10 ml methanol was treated with 1 ml of a conc. sodium hydroxide solution for 1 hour. After neutralization with IR-120 (H<sup>+</sup>), the solution was evaporated to give a residue which was treated overnight with 4,4-dimethoxytrityl chloride (1.6 g, 4.8 mmol) in 10 ml of CH<sub>2</sub>Cl<sub>2</sub> containing DBU (1.5 ml, 10 mmol). The reaction product

was purified over a silica gel column, using an heptane-ethyl acetate solvent gradient, to give 870 mg of 2-(4,4-dimethoxytrityloxymethyl)-cyclopentanol **9** as a foam (52%, 2 steps).  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  (ppm): 7.49-6.8 (m, 13H, Arom.); 4.36 (t, 1H, H-1); 3.75 (s, 6H, OMe); 3.4-3.1 (m, 2H,  $\text{OCH}_2$ ); 2.28-2.16 (m, 1-H, H-2); 1.8-1.32 (6H, m,  $3\times\text{CH}_2$ ). Analysis: Calc. for  $\text{C}_{27}\text{H}_{30}\text{O}_4$ : C 77.48, H 7.23; Found: C 77.52, H 7.59.

(1*S*,2*R*) 2-(4,4-Dimethoxytrityloxymethyl)-cyclopentanyl *p*-nitrobenzoate **10**: To a solution of **9** (470 mg, 0.94 mmol) in 10 ml THF were added successively triphenylphosphine (1.09 g, 4.2 mmol) and diethyl azodicarboxylate (790  $\mu\text{l}$ , 5 mmol). Then, after cooling to  $0^\circ\text{C}$ , *p*-nitrobenzoic acid (693 mg, 4.2 mmol) was added. The mixture was kept overnight at room temperature. Purification of the reaction product was accomplished by silica gel column chromatography (elution with heptane/ethyl acetate, 9/1) to give 164 mg of 2-(4,4-dimethoxytrityloxymethyl)-cyclopentanyl *p*-nitrobenzoate **10** as an amorphous powder (26%).  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  (ppm): 8.19-8.15 (m, 4H, Arom.); 7.32-7.21 (m, 9H, Arom.); 6.8-6.75 (m, 4H, Arom.); 5.3 (t, 1H, H-1); 3.73 (s, 6H, OMe); 3.07 (m, 2H,  $\text{OCH}_2$ ); 2.48 (m, 1-H, H-2); 2.05-0.9 (6H, m,  $3\times\text{CH}_2$ ).  $^{13}\text{C}$  nmr ( $\text{CDCl}_3$ )  $\delta$  (ppm): 85.9; 80.7; 64.4; 55.2; 46; 32.4; 27.5; 23. FAB MS  $m/z$  568 ( $\text{MH}^+$ ). Analysis: Calc. for  $\text{C}_{34}\text{H}_{33}\text{NO}_7$ : C 71.94, H 5.86, N 2.47; Found: C 72.25, H 6.13, N 2.29.

(1*S*,2*R*) 2-(4,4-Dimethoxytrityloxymethyl)-cyclopentanol **11**: After a 4 hour treatment of a solution of compound **10** (286 mg, 0.5 mmol) in 10 ml THF with 1 ml of 10% NaOH aqueous solution, the reaction mixture was carefully concentrated, diluted with ethyl acetate. The organic phase was washed with water, dried over sodium sulfate and evaporated to give 139 mg of 2-(4,4-dimethoxytrityloxymethyl)-cyclopentanol **11** as an oil (73%).  $[\alpha]_D = -4^\circ$  ( $\text{CHCl}_3$ ,  $c = 1.3$ ). FAB-MS:  $m/z$  369 ( $\text{MH}^+$ ).  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  (ppm): 7.44-7.19 (m, 9H, Arom.); 6.82-6.79 (m, 4H, Arom.); 3.93 (m, 1H, H-1); 3.73 (s, 6H, OMe); 3.27-2.98 (m, 2H,  $\text{OCH}_2$ ); 2.78 (s, 1H, OH); 2.11 (m, 1H, H-2); 1.84-1.42 (6H, m,  $3\times\text{CH}_2$ ).  $^{13}\text{C}$  nmr ( $\text{CDCl}_3$ )  $\delta$  (ppm): 86.1; 77.8; 66.7; 55.1; 47.8; 33.9; 26.6; 21.6. Analysis: Calc. for  $\text{C}_{27}\text{H}_{30}\text{O}_4$ , 0.5  $\text{H}_2\text{O}$ : C 75.85, H 7.31; Found: C 75.66, H 7.52.

(1*S*,2*R*) 2-Cyanoethyl-[2'-(4,4-dimethoxytrityloxymethyl)]-cyclopentanyl *N,N*-diisopropylphosphoramidite **12**: To a solution of **11** (164 mg, 0.33 mmol) in 1.2 ml of dichloromethane containing *N,N*-diisopropylethylamine (150  $\mu\text{l}$ , 0.9 mmol) was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (100  $\mu\text{l}$ , 0.45 mmol). The reaction mixture was stirred at room temperature for 1 h. Then it was diluted with

dichloromethane and the resulting solution washed with aqueous sodium bicarbonate. The reaction product was purified on a short silicagel column to give 131mg of amorphous 2-cyanoethyl-1'-[2'-(4,4-dimethoxytrityloxymethyl)]-cyclopentanyl *N,N*-diisopropylphosphoramidite **12** (61%). FAB MS *m/z* 547 (MH<sup>+</sup>). <sup>31</sup>P nmr (CDCl<sub>3</sub>) δ (ppm): 147.9-147.3

### ACKNOWLEDGEMENTS

This work was supported by research grants from MRES (ACC-5 SV) and ARC.

### REFERENCES

- 1 (a) Lindahl, T.; Sedgwick, B.; Sekiguchi, M; Nakabeppu, Y. *Annu. Rev. Biochem.* **1988**, *57*, 133-157. (b) Dodson, M. L.; Michaels, M. L.; Lloyd, R. S. *J. Biol. Chem.* **1994**, *52*, 32709-32712. (c) Lloyd, R. S.; Linn, S. In *Nucleases*; Linn, S.; Lloyd, R. S.; Roberts, R. J. eds; CSHL Press 1993, 2, pp 263-313.
- 2 Takeshita, M.; Chang, C.-N.; Johnson, F.; Will, S.; Grollman, A. *J. Biol. Chem.* **1987**, *262*, 10171-10179.
- 3 For preparations of (1*S*,2*R*)-, (1*S*,2*S*)- and (1*R*,2*S*) 2-(hydroxymethyl)cyclopentanol see: (a) Buisson, D.; Azerad, R. *Tetrahedron Asymmetry* **1996**, *7*, 9-12. (b) Weidner, J.; Theil, F.; Schick, H. *Tetrahedron Asymmetry* **1994**, *5*, 751-754. Inoguchi, K.; Fujie, N.; Yoshikawa, K.; Achiwa, K. *Chem. Pharm. Bull* **1992**, *40*, 2921-2926. (d) Fang, C.; Ogawa, T.; Suemune, H.; Sakai, K. *Tetrahedron Asymmetry* **1991**, *2*, 389-398.
- 4 (a) Burlina, F.; Favre, A.; Fourrey, J.-L.; Thomas, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 247-250. (b) Burlina, F.; Favre, A.; Fourrey, J.-L.; Thomas, M. *J. Chem. Soc., Chem. Commun.* **1996**, 1623-1624.
- 5 (a) Burlina, F.; Clivio, P.; Fourrey, J.-L.; Riche, C.; Thomas, M. *Tetrahedron Lett.* **1994**, *35*, 8151-8152. (b) Grieco, P. A.; Henry, K. J.; Nunes, J.J.; Matt Jr, J. E. *J. Chem. Soc., Chem. Commun.* **1992**, 368-370. (c) Lubineau, A.; Augé, J.; Lubin, N. *Tetrahedron Lett.* **1991**, *32*, 7529-7530.
- 6 Conole, G.; Mears, R. J.; De Silva, H.; Whiting, A. *J. Chem. Soc. Perkin Trans 1* **1995**, 1825-1836.

received 9/4/98

accepted 11/23/98