Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4051–4054

Synthesis of oligodeoxynucleotides containing a single diastereoisomer of α-(N^2-2′-deoxyguanosinyl)-*N*-desmethyltamoxifen

Y. R. Santosh Laxmi, Naomi Suzuki, Sung Yeon Kim and Shinya Shibutani*

Laboratory of Chemical Biology, Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794-8651, USA

Received 21 April 2004; revised 13 May 2004; accepted 14 May 2004
Available online 8 June 2004

Abstract—A phosphoramidite chemical synthesis of oligodeoxynucleotides containing a diastereoisomer of (*E*)-α-(N^2-deoxyguanosinyl)-*N*-desmethyltamoxifen, a major tamoxifen (TAM)-derived DNA adduct in animal and women treated with TAM, was described. The site-specifically modified oligodeoxynucleotide can be used for mutagenesis, DNA repair, and 3D structural studies and also as standard for quantitative analysis of TAM-DNA adducts in animal and human.

Tamoxifen (TAM, **1a**; [*E*-1-[4-{2-(dimethylamino)-ethoxy}-phenyl]-1,2-diphenyl]-butene) is widely used in the endocrine therapy of breast cancer and used as a prophylactic agent for women at high risk of developing breast cancer.[1] However, administration of TAM is associated with an increased risk of endometrial cancer in women treated with TAM.[2] TAM is metabolized by phase I enzymes to *N*-desmethyltamoxifen (*N*-desTAM, **1c**), 4-hydroxytamoxifen, tamoxifen *N*-oxide, and α-hydroxytamoxifen (α-OHTAM, **1b**).[3] *N*-desTAM is further metabolized to α-hydroxy-*N*-desTAM (**1d**). α-Hydroxylated TAM metabolites, **1d** and **1b**, are O-sulfonated by rat and human hydroxysteroid sulfotransferases and react with the exocyclic amino group of guanine in DNA via a short-lived carbocation intermediate, resulting in the formation of two (*E*) (fr-1 and fr-2) and two (*Z*) (fr-3 and fr-4) diastereoisomers of the α-(N^2-deoxyguanosyl)-*N*-desmethyltamoxifen adducts (dG-N^2-*N*-desTAM **2b**, the structures in Fig. 1) and α-(N^2-deoxyguanosyl)tamoxifen adducts (dG-N^2-TAM, **2a**), respectively. In fact, (*E*)-dG-N^2-*N*-desTAM and (*E*)-dG-N^2-TAM adducts have been detected as major DNA adducts in rodents, monkey and women treated with TAM.[3] In the present study, we describe a phosphoramidite chemical synthesis of (*E*)-dG-N^2-*N*-desTAM-modified oligodeoxynucleotides that can be utilized for exploring biological properties and three-dimensional structure of dG-N^2-*N*-desTAM adducts. When following a protocol previously established for the preparation of dG-N^2-TAM-modified oligomer,[4] the synthesis of dG-N^2-*N*-desTAM-modified oligomers was not successful. Several modifications, including protection and deprotection of the secondary amino group of *N*-desTAM and deletion of the capping step during automated oligonucleotide synthesis, were required for preparation of large quantities of (*E*)-dG-N^2-*N*-desTAM-modified oligodeoxynucleotide.

The secondary amino group of (*E*)-α-OH-*N*-desTAM **1d** was protected with a benzyloxycarbonyl moiety[5] (Scheme 1). The protected (*E*)-α-NH_2-*N*-desTAM **5**[12] was synthesized from the compound **3**[5] using the Mitsunobu reaction[5] followed by hydrolysis.[7] The DMT-derivative of 2-fluoro-(O^6-trimethylsilylethyl)-2′-deoxyinosine **6**[8] was coupled with the (*E*)-α-NH_2-*N*-desTAM protected at the aminoethoxy functionality **5** to produce compound **7**[12] (Scheme 1). Hydrogenation using palladium hydroxide[9] (10%) at 50 psi for 48 h successfully deprotected the benzyloxycarbonyl group to give the free secondary amino compound **8**.[12] dG-N^2-*N*-desTAM phosphoramidite derivative **9**[12] was prepared using diisopropylammonium tetrazolide.[10]

Keywords: *N*-Desmethyltamoxifen; Oligodeoxynucleotide; Phosphoramidite; DNA adduct.
* Corresponding author. Tel.: +1-631-444-8018; fax: +1-631-444-3218; e-mail: shinya@pharm.sunysb.edu

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.05.030

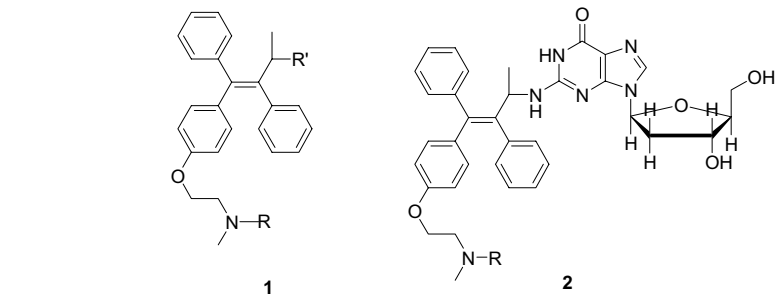
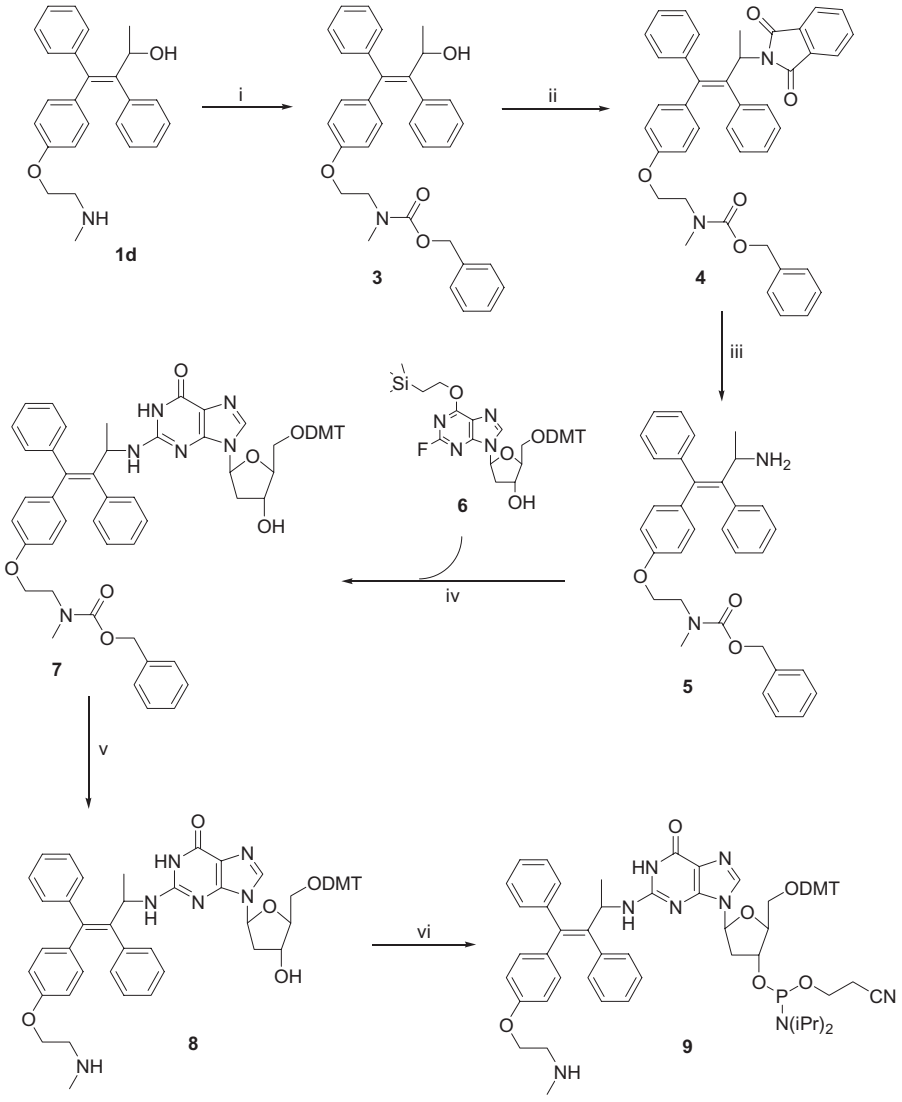


Figure 1. Structures of (*E*)- and (*Z*)-forms of TAM metabolites and TAM-DNA adducts.



Scheme 1. Synthetic protocol for dG-N^2-*N*-desTAM-modified oligodeoxynucleotides. Reagents and conditions: (i) $ClCO_2CH_2Ph$, Et_3N, THF, rt, 90%, (ii) phthalimide, TPP, DEAD, rt, 48%, (iii) $MeNH_2$ in EtOH, reflux, 94%; (iv) DMSO, Et_3N, 75 °C, 73%; (v) $Pd(OH)_2$ 10 mol %, ethylacetate, Et_3N, 50 psi, 78%; (vi) diisopropylammonium tetrazolide, $P(N(i\text{-}Pr)_2)_2)OCH_2CH_2CN$, 66%.

The preparation of the dG-N^2-*N*-desTAM-modified oligodeoxynucleotide using the phosphoramidite precursor **9**, by general method in the DNA synthesizer, gave more than 90% of oligomer containing an acetyl group. It was not possible to remove the acetyl group at the secondary amino group of the side chain, which was

obtained during the capping process, after the synthesis of the oligomer in the DNA synthesizer. Therefore, the capping step was avoided in the synthesizer. The desired dG-N^2-*N*-desTAM-modified oligodeoxynucleotides was obtained by HPLC purification.[4] This method successfully gave the required oligodeoxynucleotide containing the dG-N^2-*N*-desTAM. The coupling efficiency for 0.25 μmol scale synthesis was >85%, and resulted in good yields of the oligomers.

The oligodeoxynucleotides (5′-GAGGTGCXTGTTTGT, where X is dG-N^2-*N*-desTAM as a single diastereoisomer) were prepared by automated chemical synthesis. The sequence of the 15-mer oligomer was selected from codons 271–275 of *P53* mutational hotspots. When the (*E*)-dG-N^2-*N*-desTAM phosphoramidite **9** was used, two diastereoisomeric oligomers containing fr-1 or fr-2 of (*E*)-dG-N^2-*N*-desTAM were separated by HPLC; the retention times of purified fr-1 and fr-2 of the modified oligomers were 31.9 and 37.6 min, respectively. The molecular weight of dG-N^2-*N*-desTAM-modified oligomers was measured by LC/MS/MS in negative ion mode. The spectra of 15-mer oligomer containing a fr-1 or fr-2 of (*E*)-dG-N^2-*N*-desTAM (5′-GAGGTGCXTGTTTGT, where X is dG-N^2-*N*-desTAM) exhibited an ion at m/z 5025, identifying

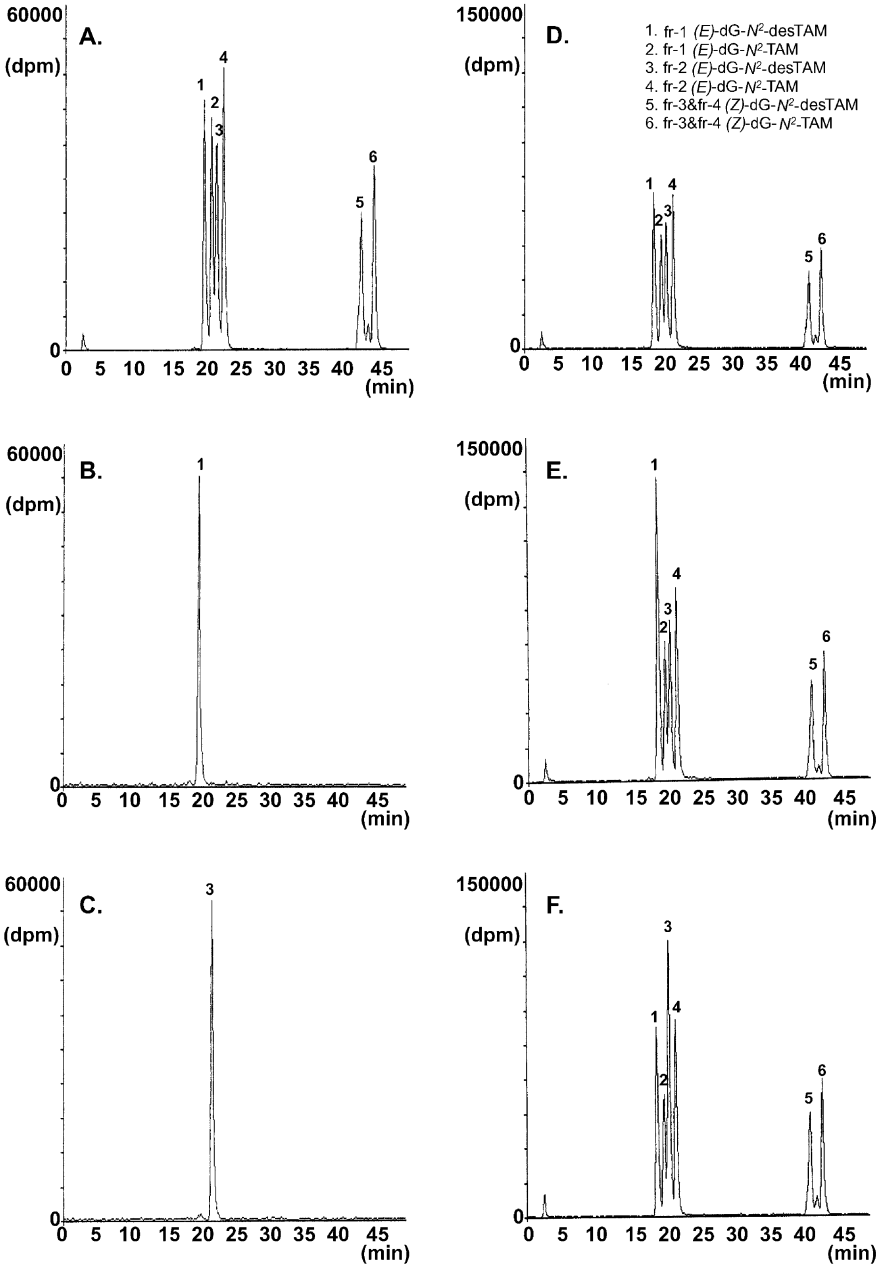


Figure 2. ^{32}P-Postlabeling/HPLC analysis of dG-N^2-*N*-desTAM-modified oligodeoxynucleotide. Standards of a mixture of (*E*)-forms (fr-1 and fr-2) and (*Z*)-forms (a mixture of fr-3 and fr-4) of dG_3-N^2-*N*-desTAM (*Z*)-dG_3-N^2-TAM were labeled with ^{32}P and subjected on HPLC (A). 15-mer oligodeoxynucleotides (50 ng, 5′-GAGGTGCXTGTTTGT, where X is dG-N^2-*N*-desTAM) containing a single (*E*)-isoform (fr-1, B; fr-2, C) of dG-N^2-*N*-desTAM were digested enzymatically, and labeled with ^{32}P. Standards (D) were co-chromatographied with a product from oligomer containing fr-1 (E) or fr-2 (F) of dG-N^2-*N*-desTAM.

the molecular mass as 5026 Da. Several (*E*)-dG-N^2-*N*-desTAM-modified oligomers having different sequence context have been successfully synthesized, and these modified oligomers were also resolved into two diastereoisomeric oligomers by HPLC.

^{32}P-Postlabeling/HPLC analysis developed recently in our laboratory[11] can also be used to confirm the incorporation of dG-N^2-*N*-desTAM into oligomers. As shown in Figure 2A, standards of (*E*)- (fr-1 and fr-2) and (*Z*)- (a mixture of fr-3 and fr-4) isomers of $dG_{3/P}$-N^2-*N*-desTAM and dG_3-N^2-TAM can be resolved in 50 min. The retention times of a DNA adduct from fr-1 and fr-2 of the modified oligomers were 19.8 min (Fig. 2B) and 20.5 min (Fig. 2C), respectively. When the DNA adduct from oligomer containing fr-1 (Fig. 2E) or fr-2 (Fig. 2F) was co-injected with the authentic standards (Fig. 2D), the products were identified as fr-1 and fr-2 of (*E*)-$dG_{3/p}$-N^2-*N*-desTAM, respectively.

Thus, phosphoramidite chemical synthesis allows the preparation of substantial quantities of oligomers containing dG-N^2-*N*-desTAM adduct(s) in virtually any sequence context. These dG-N^2-*N*-desTAM-modified oligomers will be used for mutagenesis, DNA repair studies, 3D NMR structural and crystallographic studies. Such modified oligomers can also be used as standards for ^{32}P-postlabeling analysis to quantify dG-N^2-*N*-desTAM-DNA adducts in animal and human.

Acknowledgements

This research was supported by a Grant ES09418 from the National Institute of Environmental Health Sciences. We thank Mrs. Cecilia M. Torres for synthesis of modified oligodeoxynucleotides by automated DNA synthesizer and Mr. Robert Rieger for mass measurement.

References and notes

1. Jordan, V. C. *Br. J. Pharmacol.* **1993**, *110*, 507–517.
2. (a) van Leeuwen, F. E.; Benraadt, J.; Coebergh, J. W. W.; Kiemeney, L. A. L. M.; Diepenhorst, F. W.; van den Belt-Dusebout, A. W.; van Tinteren, H. *Lancet* **1994**, *343*, 448–452; (b) Fischer, B.; Costantino, J. P.; Wickerham, L.; Redmond, C. K.; Kavanah, M.; Cronin, W. M.; Botel, V.; Robidoux, A.; Dimitrov, N.; Atkins, J.; Daly, M.; Wieand, S.; Tan-Chiu, E.; Ford, L.; Wormark, N., et al. *J. Natl. Cancer Inst.* **1998**, *90*, 1371–1388.
3. Kim, S. Y.; Suzuki, N.; Santosh Laxmi, Y. R.; Shibutani, S. *Drug Metab. Rev.* **2004**, *36*, 199–218.
4. Santosh Laxmi, Y. R.; Suzuki, N.; Dasaradhi, L.; Johnson, F.; Shibutani, S. *Chem. Res. Toxicol.* **2002**, *15*, 218–225.
5. Kitagawa, M.; Ravindernath, A.; Suzuki, N.; Rieger, R.; Terashima, I.; Umemoto, A.; Shibutani, S. *Chem. Res. Toxicol.* **2000**, *13*, 761–769.
6. Mitsunobu, O.; Wada, M.; Sano, T. *J. Am. Chem. Soc.* **1971**, *94*, 679–680.
7. Motawia, M. S.; Wengel, J.; Abdel-Megid, A. E. S.; Pedersen, E. B. *Synthesis* **1989**, 384–387.
8. DeCorte, B. L.; Tsarouhtsis, D.; Kuchimanchi, S.; Cooper, M. D.; Horton, P.; Harris, C. M.; Harris, T. M. *Chem. Res. Toxicol.* **1996**, *9*, 630–637.
9. Beaulieu, P. L.; Schiller, P. W. *Tetrahedron Lett.* **1988**, *29*, 2019–2022.
10. Mcgee, D. P. C.; Cook, P. N.; Guinosso, C. J. PCT. Int. Appl., WO 9402501, 1994; p 85.
11. Shibutani, S.; Suzuki, N.; Santosh Laxmi, Y. R.; Schild, L. J.; Divi, R. L.; Grollman, A. P.; Poirier, M. C. *Cancer Res.* **2003**, *63*, 4402–4406.
12. Spectral data of new compounds:
5: FAB mass: *m/z* 506 (M^+). ^{1}H NMR ($CDCl_3$) δ ppm: 1.13 (d, 3H, $J = 6.9$ Hz, HC–CH_3); 3.01 (s, 3H, N(CH_3)); 3.57–3.61 (m, 2H, N–CH_2); 3.90–4.01 (m, 2H, O–CH_2); 4.10 (q, 1H, $J = 6.96$ Hz, $NH_2HC(CH_3)$); 6.46–6.55 (m, 2H, H-3,5 of CC_6H_4O); 6.79–6.85 (m, 2H, H-2,6 of CC_6H_4O); 7.20–7.43 (m, 15H, phenyls). ^{13}C NMR ($CDCl_3$) δ ppm: 22.8 (HC–CH_3); 35.8 (N(CH_3)); 48.7 ($NH_2HC(CH_3)$); 47.9 and 48.7 (N–CH_2); 65.7 and 66.1 (O–CH_2); 66.9 (O–CH_2Ph); 113.1, 126.2, 126.6, 127.4, 127.6, 127.7, 127.8, 128.2, 128.3, 129.2, 130.8, 131.0, 134.9, 136.6, 138.4, 138.7, 142.2, 144.1, 156.1, 156.2, 156.4.
7: FAB mass: *m/z* 1059 (M^++1). ^{1}H NMR (CD_3OD) δ ppm: 1.39 (m, 3H, HC–CH_3); 2.95 (s, 3H, N(CH_3)); 3.53–3.61 (m, 2H, N–CH_2); 3.82–3.98 (m, 2H, O–CH_2), 5.05–5.12 (m, 2H, O–CH_2–Ph); 5.15–5.24 (m, 1H, N–CH–CH_3), 6.39–7.52 (m, 33H, aromatic and C1′-H of sugar moiety); 7.84–7.85 (m, 1H, H at C-8 of dG), sugar moiety: 2.40–2.56 (m, 2H, 2′-CH_2); 3.20–3.45 (m, 2H, 5′CH_2–OH); 3.78 (s, 6H, OCH_3); 4.15–4.22 (m, 1H, 4′-CH); 4.53–4.62 (m, 1H, 3′-CH).
8: FAB mass: *m/z* 925 (M^++1). ^{1}H NMR (CD_3OD) δ ppm: 1.34–1.36 (m, 3H, HC–CH_3); 2.44–2.49 (2s, 3H, N(CH_3)); 2.85–2.98 (m, 2H, N–CH_2); 3.81–3.95 (m, 2H, O–CH_2), 5.06–5.21 (m, 1H, N–CH–CH_3), 6.36–7.49 (m, 33H, aromatic and C1′-H of sugar moiety); 7.84–7.85 (m, 1H, H at C-8 of dG), sugar moiety: 2.62–2.76 (m, 2H, 2′-CH_2); 3.32–3.48 (m, 2H, 5′CH_2–OH); 3.70 (s, 3H, OCH_3); 3.73 (s, 3H, OCH_3); 4.12–4.21 (m, 1H, 4′-CH); 4.48–4.58 (m, 1H, 3′-CH).
9: ^{1}H NMR (CD_3OD) δ ppm: 1.04–1.16 (m, 12H, 2[$(CH_3)_2CH$]); 1.32–136 (m, 3H, CHCH_3); 2.31–2.39 (m, 2H, C2′H); 2.43–2.52 (m, 2H, CH_2CN); 2.55–2.61 (m, 3H, N(CH_3)); 2.63–2.76 (m, 2H, NCH_2); 3.34–3.54 (m, 4H, P–O–CH_2, 2NCH(CH_3)$_2$); 3.57–3.68 (m, 2H, 5′CH_2OH); 3.70–3.75 (m, 6H, OCH_3); 3.86–3.95 (m, 2H, OCH_2); 4.12–4.21 (m, 1H, 4′CH of sugar moiety); 4.48–4.58 (m, 1H, 3′CH of sugar moiety); 5.11–5.21 (m, 1H, HNCHCH_3); 6.33–7.49 (m, 29H, aromatic and 1′CH); 7.81–7.85 (m, 1H, H at C8 of dG). ^{31}P NMR (CD_3OD) δ ppm: 149.84, 150.27, 150.36.