Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 741–744
Bioorganic & Medicinal Chemistry Letters

Synthesis of a technetium-99m labeled tricyclic ganciclovir analog for non-invasive reporter gene expression imaging

Yi Zhang, Jing Lin and Dongfeng Pan*

The Department of Radiology, University of Virginia, Charlottesville, VA 22908, USA

Received 19 September 2006; revised 24 October 2006; accepted 25 October 2006
Available online 28 October 2006

Abstract—A potential radiopharmaceutical and HSV1-TK substrate, 3-((1,3-dihydroxypropan-2-yloxy)-methyl)-6-(4-(3-((2-mercaptoethyl)(2-(2-mercaptoethyl-amino)-ethyl)amino)propoxy)phenyl)-3*H*-imidazopurin-9(5*H*)-one-oxo-technetium(V), was synthesized via a converging approach and its chemical structure was comparatively characterized with a non-radioactive analog. The final radiochemical purity and yield were 97 and 73%, respectively.

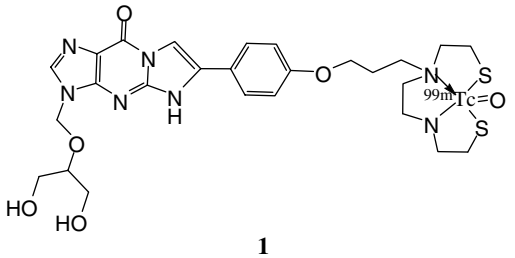
Non-invasive nuclear imaging of the Herpes simplex virus type-1 thymidine kinase (HSV1-TK) expression has received considerable attention due to its potential clinical application in gene therapy.[1–4] Current nuclear reporter probes of HSV1-TK include fluorine and iodine isotope labeled pyrimidine nucleoside derivatives[5–9] and acycloguanosine derivatives.[10,11] However, their use in routine procedures is hampered by either the limited supply of the cyclotron-produced isotopes, F-18 and I-123/124, or the suboptimal imaging characteristics of I-131, which yields high and multiple emission energies. There are currently interests for novel tracers suitable for routine application.

Miscellaneous potential HSV-TK subjects have been studied as either imaging or antiherpes therapeutic agent. For example, a series of tricyclic analogs of ACV and GCV have been synthesized and evaluated as potential fluorescent cellular imaging probes for herpes virus assay and some of them demonstrated the similar activity and selectivity to herpes simplex virus type I as ACV or GCV.[12–14]

In our exploration of the Tc-99m labeled TK substrates for nuclear gene imaging, tricyclic ganciclovir (TGCV) was selected as a template because it seems to be sufficient for HSV-TK interaction and the modifications at the 4-position of the phenyl ring are permitted (Fig. 1). Radiometal labeling was accomplished through a neutral bisaminoethanethiol (N_2S_2) based chelating moiety.

In this communication, we report the synthesis and characterization of 3-((1,3-dihydroxypropan-2-yloxy) methyl)-6-(4-(3-((2-mercaptoethyl)(2-(2-mercaptoethyl-amino)ethyl)amino)-propoxy)phenyl)-3*H*-imidazo[1,2-f]-purin-9(5*H*)-one-oxo-[Tc-99m] **1**. To characterize the chemical structure of the Tc-99m labeled probe, a non-radioactive mimic compound, 3-((1,3-dihydroxypropan-2-yloxy)methyl)-6-(4-(3-((2-mercaptoethyl)(2-(2-mercaptoethylamino)ethyl)amino)propoxy)-phenyl)-3*H*-imidazo-[1,2-f]purin-9(5*H*)-one-oxo-[Re] **2**[15] is synthesized for NMR and MS analysis and HPLC identification.

At first, the tricyclic fragment **3**[16] was synthesized (Scheme 1) as follows. Coupling GCV **4** and phenacyl bromide **5** yielded the tricyclic TGCV **6**,[12] followed by

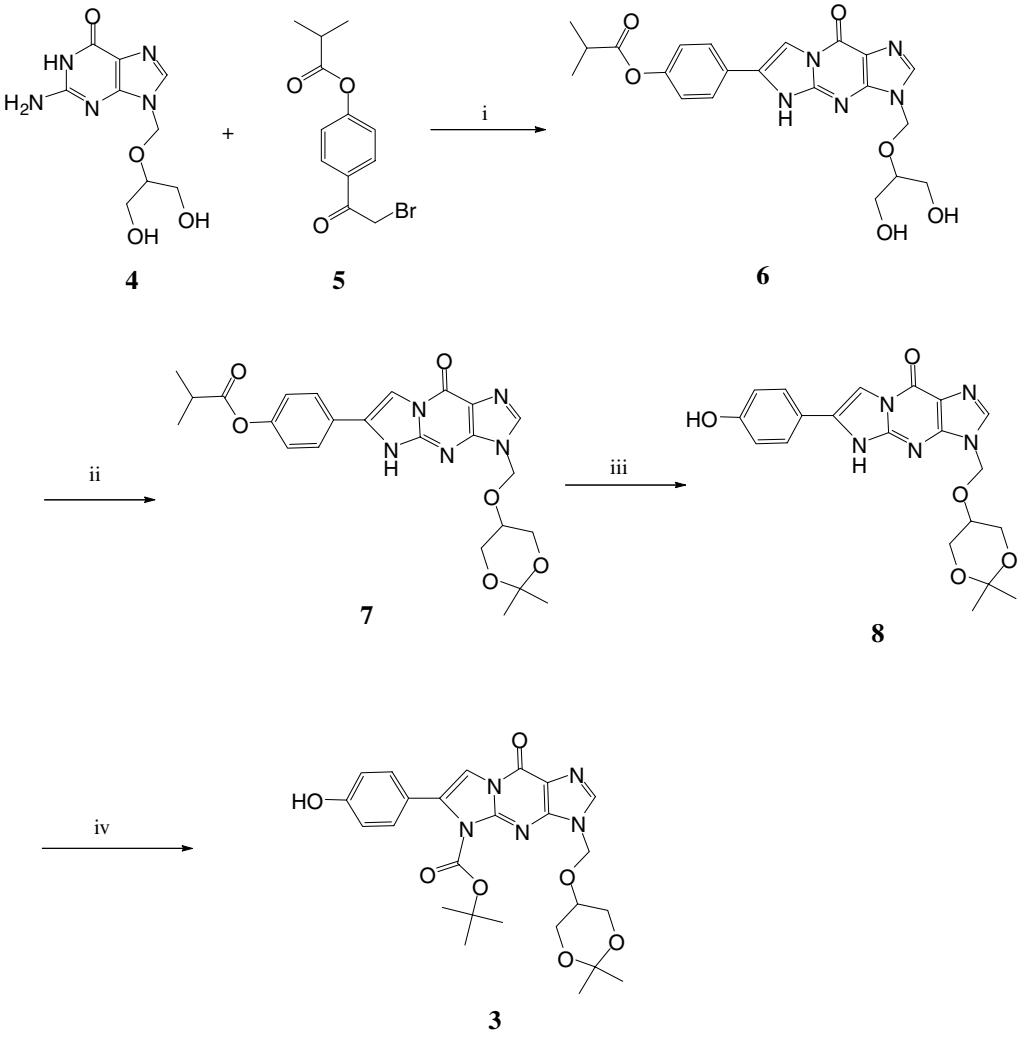


1

Figure 1. Technetium-99m complex of tricyclic ganciclovir (TGCV).

Keywords: Nuclear imaging tracer; HSV1-TK substrate; Technetium-99m.
* Corresponding author. Tel.: +1 434 243 2893; fax: +1 434 924 9435; e-mail: dp3r@virginia.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.10.077



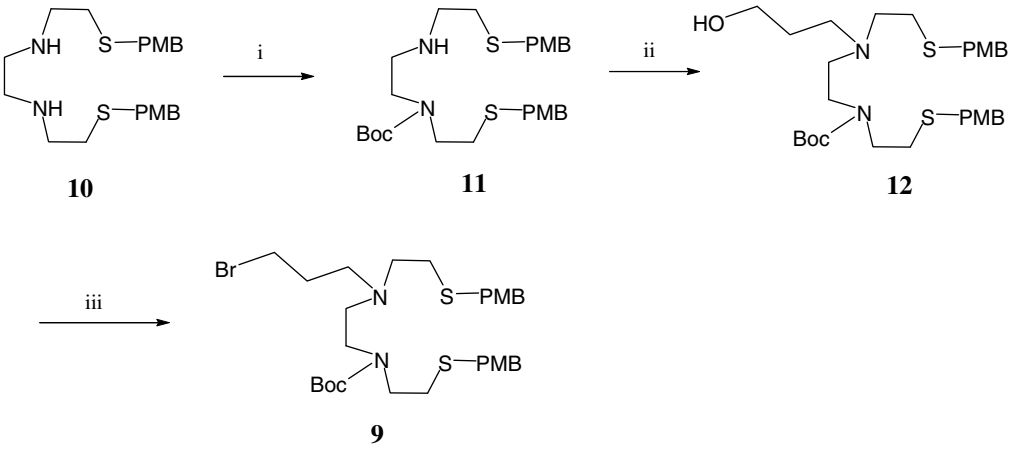
Scheme 1. Reagents and condition: (i) NaH/DMF, 86%; (ii) DMF, 2-methoxypropene, TsOH·H_2O, 74%; (iii) NH_3–MeOH, 52%; (iv) Boc_2O, 1,4-dioxane, aq Na_2CO_3, 78%.

protecting the free hydroxyls with 2-methoxypropene in presence of TsOH to give isopropylidene ketal **7**.[17] Removal of the isobutanoyl group of **7** with saturated methanolic ammonia gave phenol **8**.[18] The secondary amino group at position 5 was protected with *t*-Boc by mixing compound **8** with di-*tert*-butyl dicarbonate in 1,4-dioxane and aqueous Na_2CO_3.

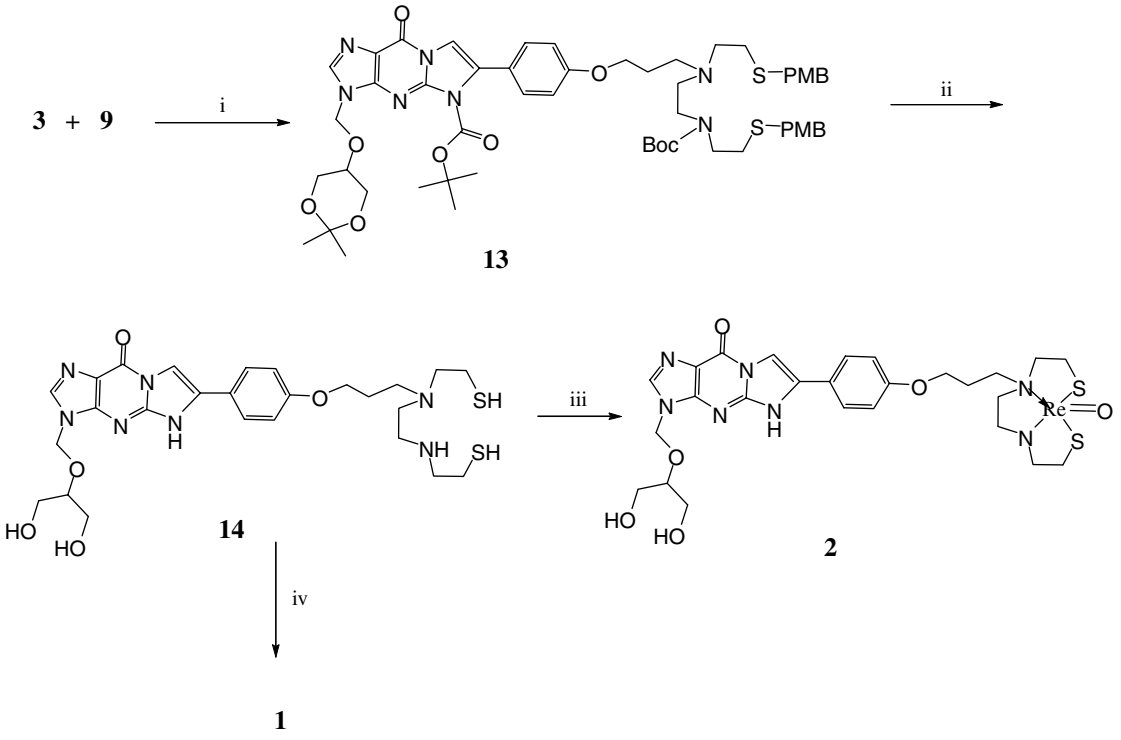
The synthesis of radiometal-chelating moiety **9**[19] started from *N*,*N*′-bis-[2-(4-methoxy-benzylsulfanyl)-ethyl]-ethylenediamine **10**, which was synthesized as described previously (Scheme 2).[20] After blocking one of the secondary amino groups of **10** with *t*-Boc to produce **11**,[21] the monoalkylated **12**[22] was obtained by coupling of **11** with 3-bromo-1-propanol. The subsequent bromination of **12** with NBS/triphenylphosphine yielded **9** (see Scheme 3).

Coupling of TGCV derivative **3** and chelator fragment **9** produced **13**,[23] the precursor of the target radiotracer **1**. After deprotection of **13** with $Hg(OAc)_2$ in TFA, to the crude intermediate **14** (50 μg) in aqueous methanol in the presence of Sn-glucoheptonate was added 2.1 mCi of ^{99m}Tc pertechnetate in PBS buffer, and 1.5 mCi of the target radiochemical **1** was obtained after HPLC purification with a C18 reversed-phase Econosphere column (250 × 10 mm, 30% CH_3CN in H_2O, 3 mL/min, 254 nm). The radiotracer was collected with retention time at 10.38 min and radiochemical yield of 73%. To characterize the chemical structure of the radiochemical **1**, a rhenium conjugated analog **2** was synthesized by adding tetrabutylammonium tetrachlorooxorhenate(V) into compound **14** in methanol and stirring for 12 h. The rhenium conjugated **2** was purified by flash chromatography and characterized with ^{1}H NMR and mass spectrum. The characterization of Tc-99m tracer **1** was carried out by co-injection with rhenium analog **2** using reverse phase HPLC.

In conclusion, we have successfully synthesized a Tc-99m labeled acycloguanosine derivative **1**, a potential HSV1-TK substrate for nuclear gene imaging. In vitro cell uptake assays of the compound are currently in progress.



Scheme 2. Reagents and condition: (i) Boc_2O, 1,4-dioxane, aq Na_2CO_3, 74%; (ii) acetonitrile, 3-bromo-1-propanol, DIEA, 71%; (iii) NBS, PPh_3, DMF, 64%.



Scheme 3. Reagents and condition: (i) $CHCl_3$, aq Na_2CO_3, $Bu_4N^+Br^-$, 81%; (ii) $Hg(OAc)_2$/TFA, H_2S; (iii) $(Bu_4N)^+(ReOCl_4)^-$, 58%; (iv) [^{99m}Tc]$NaTcO_4$, Sn-gluceptate, 73%.

Acknowledgments

This work was supported in part by Grants from DOD PC010112, the Nihon Medi-Physics Co. Ltd (NMP), and the Department of Radiology, University of Virginia.

References and notes

1. Yaghoubi, S. S.; Barrio, J. R.; Namavari, M.; Satyamurthy, N.; Phelps, M. E.; Herschman, H. R.; Gambhir, S. S. *Cancer Gene Ther.* **2005**, *12*, 329.
2. Min, J. J.; Lyer, M.; Gambhir, S. S. *Eur. J. Nucl. Med. Mol. Imaging* **2003**, *30*, 1547.
3. Shah, K.; Jacobs, A.; Breakefield, X. O.; Weissleder, R. *Gene Ther.* **2004**, *11*, 1175.
4. Lyer, M.; Gambhir, S. S. *Lung Biol. Health Dis.* **2005**, *203*, 171.
5. Mercer, J. R.; Xu, L. H.; Knaus, E. E.; Wiebe, L. I. *J. Med. Chem.* **1989**, *32*, 1289.
6. Tjuvajev, J. G.; Avril, N.; Oku, T.; Sasajima, T.; Miyagawa, T.; Joshi, R.; Safer, M.; Beattie, B.; DiResta, G.; Daghighian, F.; Augensen, F.; Koutcher, J.; Zweit, J.; Humm, J.; Larson, S. M.; Finn, R.; Blasberg, R. *Cancer Res.* **1998**, *58*, 4333.
7. Morin, K. W.; Atrazheva, E. D.; Knaus, E. E.; Wiebe, L. I. *J. Med. Chem.* **1997**, *40*, 2184.
8. Wiebe, L. I.; Knaus, E. E.; Morin, K. W. *Nucleosides Nucleotides* **1999**, *18*, 1065.

9. Tjuvajev, J. G.; Finn, R.; Watanabe, K.; Joshi, R.; Oku, T.; Kennedy, J.; Beattie, B.; Koutcher, J.; Larson, S.; Blasberg, R. G. *Cancer Res.* **1996**, *56*, 4087.
10. Alauddin, M. M.; Shahinian, A.; Kundu, R. K.; Gordon, E. M.; Conti, P. S. *Nucl. Med. Biol.* **1999**, *26*, 371.
11. Alauddin, M. M.; Conti, P. S. *Nucl. Med. Biol.* **1998**, *25*, 175.
12. Goslinski, T.; Golankiewicz, B.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **2002**, *45*, 5052.
13. Golankiewicz, B.; Ostrowski, T.; Andrei, G.; Snoeck, R.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 3187.
14. Goslinski, T.; Wenska, G.; Golankiewicz, B.; Balzarini, J.; De Clercq, E. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 911.
15. Compound **2**. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.59 (m, 2H), 2.21(m, 2H), 2.50 (m, 10H), 3.13 (m, 4H), 3.42 (m, 2H), 3.61 (m, 2H), 3.81 (m, 2H), 5.63(s, 2H), 6.93, 7.44 (dd, 2H, 2H), 7.69 (s, 1H), 8.06 (s, 1H). Mass spectrum (MALDI-TOF) *m/z* 792.3 $(M+H)^+$ (theorectcal *m/z* 791.2).
16. Compound **3**. ^{1}H NMR ($CDCl^3$, 300 MHz), δ 1.30 (s, 3H), 1.34 (s, 3H), 1.55 (s, 9H), 3.62 (m, 1H), 3.70 (m, 2H), 3.83 (m, 2H), 5.48 (s, 2H), 7.23, 7.81 (dd, 2H, 2H), 7.85 (s, 1H), 7.93 (s, 1H). Mass spectrum (ESI) *m/z* 512.2142 $(M+H)^+$ ($C_{25}H_{29}N_5O_7$ requires 512.2140).
17. Compound **7**. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 1.23 (s, 1H), 1.25 (s, 1H), 1.28 (s, 6H), 2.83 (m, 1H), 3.58 (m, 1H), 3.64 (m, 2H), 3.83 (m, 2H), 5.55 (s, 2H), 7.24, 7.95 (dd, 2H, 2H), 8.07 (s, 1H), 8.22 (s, 1H). Mass spectrum (ESI) *m/z* 482.2035 $(M+H)^+$ ($C_{24}H_{28}N_5O_6$ requires 482.2034).
18. Compound **8**. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 1.27 (s, 6H), 3.56 (m, 1H), 3.60 (m, 2H), 3.83 (m, 2H), 5.54 (s, 2H), 6.85, 7.71 (dd, 2H, 2H), 7.97 (s, 1H), 8.06 (s, 1H), 9.82 (s, 1H). Mass spectrum (ESI) *m/z* 412.1615 $(M + H)^+$ ($C_{20}H_{22}N_5O_5$ requires 412.1615).
19. Compound **9**. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.44 (s, 9H), 1.88 (m, 2H), 2.54 (m, 10H), 3.17 (m, 2H), 3.32 (m, 2H), 3.45 (t, 2H), 3.67 (s, 4H), 3.79 (s, 6H), 6.85, 7.25 (dd, 2H, 2H). Mass spectrum (ESI) *m/z* 641.2078 $(M+H)^+$($C_{30}H_{46}BrN_2O_4S_2$ requires 641.2077).
20. Zhang, Y.; Dai, X.; Kallmes, D. F.; Pan, D. *Tetrahedron Lett.* **2004**, *45*, 8673.
21. Compound **11**. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.42 (s, 9H), 2.52 (m, 6H), 2.63 (m, 2H), 2.70 (m, m, 2H), 3.21 (m, 2H), 3.62 (s, 2H), 3.65 (s, 2H), 3.73 (s, 6H), 6.80, 7.20 (dd, 4H, 4H). Mass spectrum (ESI) *m/z* 521.2502 $(M+H)^+$ ($C_{27}H_{40}N_2O_4S_2$ requires 521.2502).
22. Compound **12**. ^{1}H NMR (CDCl3, 300 MHz), δ 1.42 (s, 9H), 1.61 (m, 2H), 2.51 (m, 12H), 3.22 (m, 2H), 3.66 (s, 4H), 3.76 (s, 6H), 6.83, 7.21 (dd, 2H, 2H). Mass spectrum (ESI) *m/z* 579.2920 $(M+H)^+$ ($C_{30}H_{47}N_2O_5S_2$ requires 579.2921).
23. Compound **13**. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.35 (s, 3H), 1.38 (s, 9H), 1.41 (s, 3H), 1.58 (s, 9H), 1.79 (m, 2H), 2.48 (m, 10H), 3.06 (m, 2H), 3.21 (m, 2H), 3.62 (s, 2H), 3.65 (m, 3H), 3.71 (m, 2H), 3.75 (s, 3H), 3.76 (s, 3H), 3.86 (m, 2H), 4.12 (m, 2H), 5.59 (s, 2H), 6.81 (m, 4H), 7.19 (m, 4H), 7.34, 7.48 (dd, 2H, 2H), 7.66 (s, 1H), 7.80 (s, 1H). Mass spectrum (ESI) *m/z* 1072.4879 $(M+H)^+$ ($C_{55}H_{74}N_7O_{11}S_2$ requires 1072.4882).