Bioorganic & Medicinal Chemistry Letters, Vol.3, No.8, pp. 1681-1686, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

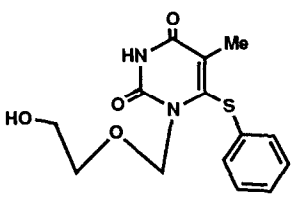
SYNTHESIS OF A POTENTIAL PHOTOAFFINITY LABELLING REAGENT FOR HIV-1 REVERSE TRANSCRIPTASE

Hiromichi Tanaka,[a]* Masanori Baba,[b] Tomomi Yamamoto,[a]
Shuichi Mori,[b] Richard T. Walker,[c] Erik De Clercq,[d] and Tadashi Miyasaka[a]
[a]*School of Pharmaceutical Sciences, Showa University, Hatanodai 1-5-8, Shinagawa-ku, Tokyo 142, Japan,*
[b]*Fukushima Medical College, Fukushima 960-12, Japan,*
[c]*School of Chemistry, University of Birmingham, Birmingham B15 2TT, United Kingdom*
[d]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium,*

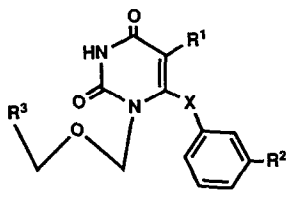
(*Received in USA* 25 *January* 1993)

Abstract An analogue (**6**) of a specific anti-HIV-1 lead, 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine (**1**: HEPT), has been synthesized with an aim to provide a tool that could be used as a specific photoaffinity labelling reagent for the HIV-1 reverse transcriptase.

In 1989, we reported the synthesis of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (**1**: HEPT) and its anti-HIV-1 (Human Immunodeficiency Virus type 1) activity.[1,2] Although HEPT can be regarded as an acyclonucleoside, its activity is highly specific to HIV-1 and required no phosphorylation of the hydroxyl group, which contrasts the known anti-HIV nucleosides such as AZT or DDI. Recently, several heterocyclic compounds were reported to show similar specificity to HIV-1.[3-7] Despite the apparent difference in their structures, HEPT and these compounds are likely to manifest their activity in a similar fashion by inhibiting the reverse transcriptase (RT) of HIV-1.

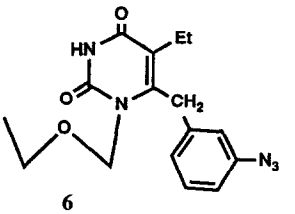


1: HEPT



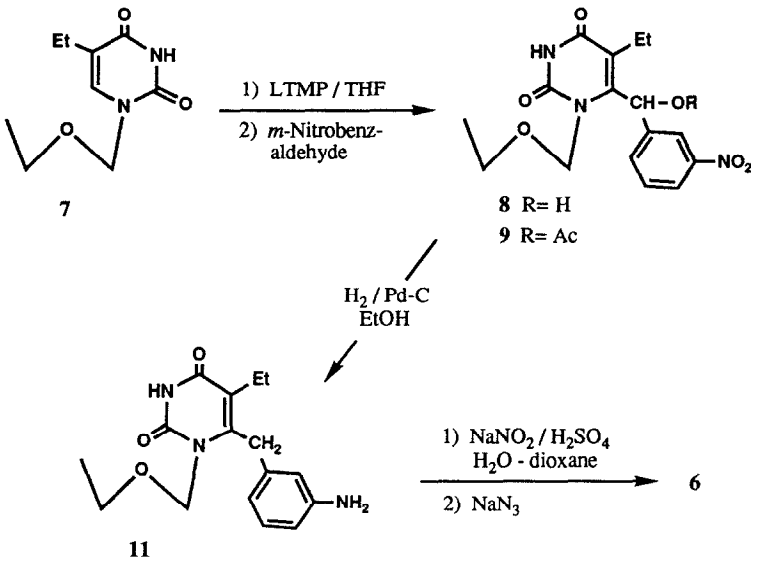
2 R^1= Me, R^2= H, R^3= CH_2OH, X= CH_2
3 R^1= R^2= Me, R^3= CH_2OH, X= S
4 R^1= Me, R^2= H, R^3= Me, X= S
5 R^1= Et, R^2= H, R^3= CH_2OH, X= S

As a result of extensive synthetic studies carried out,[8] the structure-activity relationships of HEPT analogues are fairly well defined.[9] These can be seen in the structures of compounds **2**-**5**: 1) replacement of the sulfur atom of the 6-phenylthio group with a methylene, 2) introduction of a methyl group to the *meta*-position of the phenylthio ring, 3) removal of the hydroxyl group in the acyclic structure, and 4) replacement of the 5-methyl group with a bulkier alkyl group such as an ethyl group. This evidence combined with the anticipated susceptibility of the 6-phenylthio group to nucleophilic reactions[10] led us to design compound **6** which could be used as a labelling reagent of HIV-1 RT upon photo-irradiation.[11] In this paper, synthesis of **6** and anti-HIV-1 activity of compounds involved in this study are described.



6

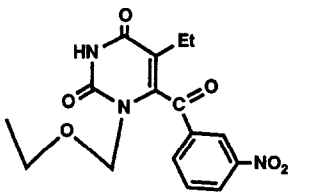
Our synthetic route to **6** is shown in Scheme 1. Lithiation at the C-6 position of 1-(ethoxymethyl)-5-ethyluracil (**7**)[8f] was carried out below −70 °C in THF by using 2.5 equiv. of lithium 2,2,6,6-tetramethylpiperidide (LTMP). The resulting C-6 lithiated species was reacted with *m*-nitrobenzaldehyde (5 equiv., below −70 °C for 1 h). Although nitro groups are generally considered to be incompatible with lithiation conditions,[12] the requisite carbinol **8** was detected as the



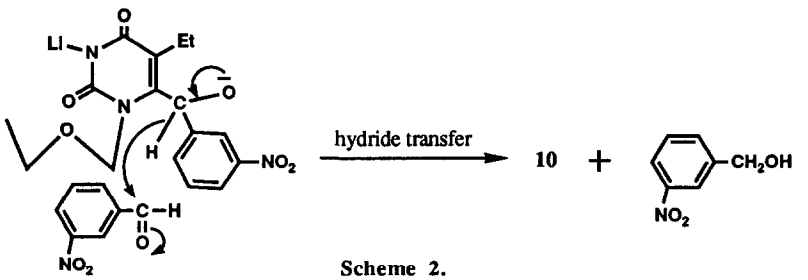
Scheme 1.

main product by TLC analysis of the reaction mixture (hexane:EtOAc = 1:1, R*f* values: *m*-nitrobenzaldehyde 0.65, **7** 0.25, and **8** 0.16). After partial purification by column chromatography, **8** was converted to its acetate **9**.[13] The overall yield of **9** from **7** was 81%.

When the preparation of **8** was performed at the higher temperature (above −60 °C), the yield of **8** decreased with concomitant formation of considerable amounts of two by-products. On the basis of ^{1}H NMR and MS spectroscopies, their structures were deduced to be the 6-(3-nitrobenzoyl) derivative **10** (R*f* 0.33)[14] and *m*-nitrobenzyl alcohol (R*f* 0.43). Formation of these two by-products would be explicable by assuming a hydride transfer from the C-6 substituted intermediate depicted in Scheme 2.

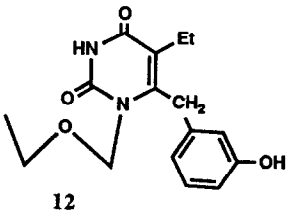


10



Scheme 2.

Catalytic hydrogenation of **9** (3.2 atm of H_2, 10% Pd-C, at 35 °C for 2 d) effected two consecutive reactions: reduction of the nitro group and then hydrogenolysis of the acetoxyl group to form the amino derivative **11** in 93% yield.[15] Finally, introduction of an azido group was carried out by nucleophilic displacement of the diazonium salt derived from **11** with sodium azide in aqueous dioxane. The desired azido derivative **6**[16] was obtained in almost quantitative yield, provided the reaction temperature was maintained below 0 °C. At a higher temperature, formation of a small amount of **12**[17] could be detected.



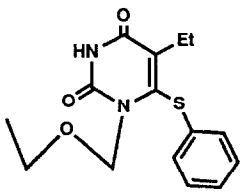
12

Anti-HIV-1 (HTLV-III$_B$ strain) activity was examined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method in MT-4 cells as reported previously.[18] As can be seen from the results summarized in Table 1, the compounds synthesized in this study are uniformly active against HIV-1. Among these, the azido derivative **6** is much more active than the original compound HEPT and its activity is almost comparable to that of the recently reported promising HEPT analogue **13**

(E-EPU).[8f,19] When the effects of **6** and E-EPU on recombinant HIV-1 RT were examined with poly(rA)·oligo(dT) as the template-primer, both compounds exhibited similar IC_{50} values (**6**, 1.6 μM *vs.* E-EPU, 1.3 μM) for the enzyme. Together with the available X-ray crystallographic analysis of HIV-1 RT,[20] compound **6** would serve as a tool to elucidate the actual site of action of HEPT analogues against the enzyme following its photochemical activation.

Table 1. Anti-HIV-1 activity of HEPT (**1**), compound **6**, and related compounds (**9-13**) in MT-4 cells.[a]

Compd	EC_{50} (μM)	CC_{50} (μM)	SI
HEPT (**1**)[b]	7.0	740	106
6	0.011±0.0	117±27	10640
9	29±13	283±49	10
10	0.21±0.05	232±1	1100
11	0.14±0.07	297±9	2120
12	0.40±0.09	201±23	503
E-EPU (**13**)	0.018±0.001	197±30	10940



13: E-EPU

[a] All data represent mean values for more than two experiments.

[b] Data taken from reference 1.

EC_{50}: effective concentration required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1.

CC_{50}: cytotoxic concentration required to reduce viability of mock-infected MT-4 cells by 50%.

SI: selectivity index (ratio of CC_{50}/EC_{50}).

Acknowledgement This work has been financially supported by a British Council Collaborative Research Project (to H. T. and R. T. W.) and by the Grant-in-Aid for Scientific Research on Priority Area (No. 03242104) from the Ministry of Education, Science, and Culture, Japan.

References and Notes

1. Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R. T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 2507.
2. Baba, M.; Tanaka, H.; De Clercq, E.; Pauwels, R.; Balzarini, J.; Schols, D.; Nakashima, H.; Perno, C.-F.; Walker, R. T.; Miyasaka, T. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1375.
3. Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Van Gelder, J.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *Nature* **1990**, *343*, 470.

4. Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C.-K.; Eckner, K.; Hattox, S.; Adams, J.; Rosenthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, R. A.; Sullivan, J. L. *Science* **1990**, *250*, 1411.
5. Goldman, M. E.; Nunberg, J. H.; O'Brien, J. A.; Quintero, J. C.; Schleif, W. A.; Freund, K. F.; Gaul, S. L.; Saari, W. S.; Wai, J. S.; Hoffman, J. M.; Anderson, P. S.; Hupe, D. J.; Emini, E. A.; Stern, A. M. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 6863.
6. Romero, D. L.; Busso, M.; Tan, C.-K.; Reusser, F.; Palmer, J. R.; Poppe, S. M.; Aristoff, P. A.; Downey, K. M.; So, A. G.; Resnick, L.; Tarpley, W. G. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 8806.
7. A nucleoside derivative having specific anti-HIV-1 activity has been reported: Balzarini, J.; Pérez-Pérez, M.-J.; San-Félix, A.; Schols, D.; Perno, C.-F.; Vandamme, A.-M.; Camarasa, M.-J.; De Clercq, E. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 4392.
8. a) Tanaka, H.; Baba, M.; Hayakawa, H.; Sakamaki, T.; Miyasaka, T.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R. T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1991**, *34*, 349. b) Tanaka, H.; Baba, M.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *ibid.* **1991**, *34*, 1394. c) Tanaka, H.; Baba, M.; Saito, S.; Miyasaka, T.; Takashima, H.; Sekiya, K.; Ubasawa, M.; Nitta, I.; Walker, R. T.; Nakashima, H.; De Clercq, E. *ibid.* **1991**, *34*, 1508. d) Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *ibid.* **1992**, *35*, 337. e) Tanaka, H.; Miyasaka, T.; Sekiya, K.; Takashima, H.; Ubasawa, M.; Nitta, I.; Baba, M.; Walker, R. T.; De Clercq, E. *Nucleosides Nucleotides* **1992**, *11*, 447. f) Tanaka, H.; Takashima, H.; Ubasawa, M.; Sekiya, K.; Nitta, I.; Baba, M.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **1992**, *35*, 4713.
9. A QSAR (Quantitative Structure-Activity Relationships) study of HEPT analogues has been reported: Hansch, C.; Zhang, L. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1165.
10. a) Tanaka, H.; Iijima, S.; Matsuda, A.; Hayakawa, H.; Miyasaka, T.; Ueda, T. *Chem. Pharm. Bull.* **1983**, *31*, 1222. b) Satoh, K.; Tanaka, H.; Andoh, A.; Miyasaka, T. *Nucleosides Nucleotides* **1986**, *5*, 461.
11. Photoaffinity labelling of the reverse transcriptase of HIV-1 with an azido analogue of Nevirapine has been reported. a) Wu, J. C.; Warren, T. C.; Adams, J.; Proudfoot, J.; Skiles, J.; Raghavan, P.; Perry, C.; Potocki, I.; Farina, P. R.; Grob, P. M. *Biochemistry* **1991**, *30*, 2022. b) Cohen, K. A.; Hopkins, J.; Ingraham, R. H.; Pargellis, C.; Wu, J. C.; Palladino, D. E. H.; Kinkade, P.; Warren, T. C.; Rogers, S.; Adams, J.; Farina, P. R.; Grob, P. M. *J. Biol. Chem.* **1991**, *266*, 14670.
12. Gschwend, H. W.; Rodriguez, H. R. In *Organic Reactions*; Dauben, W. G. Ed.; John Wiley & Sons: New York, 1979; Chapter 1; pp 1-360.

13. Physical data of **9** is as follows: mp 181-182 °C (EtOH); UV (MeOH) λ_{max} 266 nm (ε 15600), λ_{min} 235 nm (ε 5900); ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, t, 5-$CH_2C\underline{H}_3$), 1.04 (3H, t, $OCH_2C\underline{H}_3$), 2.21-2.44 (2H, overlapped with OAc, 5-$C\underline{H}_2CH_3$), 2.32 (3H, s, OAc), 3.53-3.76 (2H, m, $OC\underline{H}_2CH_3$), 5.50 and 5.76 (2H, each as d, J_{gem}= 11.0 Hz, NCH_2O), 7.05 (1H, s, C<u>H</u>OAc), 7.56-7.67 (2H, m, Ph), 8.18-8.27 (2H, m, Ph), 9.55 (1H, br, NH); FABMS *m/z* 392 (M^++H). *Anal.* Calcd for $C_{18}H_{21}N_3O_7$: C, 55.24; H, 5.41; N, 10.74. Found: C, 55.13; H, 5.42; N, 10.49.
14. Physical data of **10** is as follows: mp 127-129 °C (EtOH-Et_2O); UV (MeOH) λ_{max} 240.5 nm (ε 25000), λ_{min} 218 nm (ε 17400); ^{1}H NMR ($CDCl_3$) δ 0.76 (3H, t, 5-$CH_2C\underline{H}_3$), 0.95 (3H, t, $OCH_2C\underline{H}_3$), 2.13 (2H m, 5-$C\underline{H}_2CH_3$), 3.37 (3H, m, $OC\underline{H}_2CH_3$), 4.81 and 5.57 (2H, each as d, J_{gem}= 10.7 Hz, NCH_2O), 7.77 (1H, t, Ph), 8.27 and 8.49 (2H, each as dd, Ph), 8.58 (1H, brs, Ph), 9.50 (1H, br, NH); FABMS *m/z* 348 (M^++H). *Anal.* Calcd for $C_{16}H_{17}N_3O_6$: C, 55.33; H, 4.93; N, 12.10. Found: C, 55.43; H, 4.95; N, 11.89.
15. Physical data of **11** is as follows: mp 161-162.5 °C (EtOH); UV (MeOH) λ_{max} 245.5 (ε 11900) and 268.5 nm (ε 11300), λ_{min} 231 (ε 10300) and 259 nm (ε 10800); ^{1}H NMR ($CDCl_3$) δ 1.07 (3H, t, 5-$CH_2C\underline{H}_3$), 1.19 (3H, t, $OCH_2C\underline{H}_3$), 2.47 (2H, q, 5-$C\underline{H}_2CH_3$), 3.14 (2H, br, NH_2), 3.61 (2H, q, $OC\underline{H}_2CH_3$), 4.06 (2H, s, 6-$C\underline{H}_2$Ar), 5.12 (2H, s, NCH_2O), 6.42-6.64 (3H, m, Ph), 7.11 (1H, t, Ph), 9.01 (1H, br, NH); FABMS *m/z* 304 (M^++H). *Anal* Calcd for $C_{16}H_{21}N_3O_3$: C, 63.35; H, 6.98; N, 13.85. Found: C, 63.53; H, 7.15; N, 13.89.
16. Physical data of **6** is as follows: mp 112.5-114.5 °C (Et_2O); IR (nujol) 2140 cm^{-1} (N_3); UV (MeOH) λ_{max} 255 nm (ε 18200), λ_{min} 231 nm (ε 7800); ^{1}H NMR ($CDCl_3$) δ 1.07 (3H, t, 5-$CH_2C\underline{H}_3$), 1.18 (3H, t, $OCH_2C\underline{H}_3$), 2.46 (2H, q, 5-$C\underline{H}_2CH_3$), 3.62 (2H, q, $OC\underline{H}_2CH_3$), 4.14 (2H, s, 6-$C\underline{H}_2$Ar), 5.10 (2H, s, NCH_2O), 6.76-7.02 (3H, m, Ph), 7.34 (1H, t, Ph), 8.85 (1H, br, NH); FABMS *m/z* 330 (M^++H). *Anal.* Calcd for $C_{16}H_{19}N_5O_3$: C, 58.35; H, 5.81; N, 21.26. Found: C, 58.51; H, 5.85; N, 20.97.
17. Physical data of **12** is as follows: mp 179-180.5 °C (EtOAc); UV (MeOH) λ_{max} 271 nm (ε 12100), λ_{min} 238 nm (ε 3300); ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, t, 5-$CH_2C\underline{H}_3$), 1.15 (3H, t, $OCH_2C\underline{H}_3$), 2.40 (2H, q, 5-$C\underline{H}_2CH_3$), 3.53 (2H, q, $OC\underline{H}_2CH_3$), 4.08 (2H, s, 6-$C\underline{H}_2$Ar), 4.95 (2H, s, NCH_2O), 6.66-6.82 (3H, m, Ph), 7.23 (1H, t, Ph), 10.42 (1H, br, NH); FABMS *m/z* 305 (M^++H). *Anal.* Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20. Found: C, 63.05; H, 6.76; N, 9.05.
18. Pauwels, R.; De Clercq, E.; Desmyter, J.; Balzarini, J.; Goubau, P.; Herdewijn, P.; Vanderhaeghe, H.; Vandeputte, M. *J. Virol Methods* **1987**, *16*, 171.
19. Baba, M.; Ito, M.; Shigeta, S.; Tanaka, H.; Miyasaka, T.; Ubasawa, M.; Umezu, K.; Walker, R. T.; De Clercq, E. *Antimicrob Agents Chemother.* **1991**, *35*, 1430.
20. Kohlstaedt, L. A.; Wang, J.; Friedman, J. M.; Rice, P. A.; Steitz, T. A. *Science* **1992**, *256*, 1783.