Tetrahedron Letters 53 (2012) 5335–5337

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

# *N*-Myristoylglutamic acid derivative of 3′-fluoro-3′-deoxythymidine as an organogel

Bhupender S. Chhikara, Rakesh Tiwari, Keykavous Parang *

*Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, 41 Lower College Road, Kingston, RI 02881, USA*

ARTICLE INFO

*Article history:*
Received 21 June 2012
Revised 19 July 2012
Accepted 24 July 2012
Available online 31 July 2012

*Keywords:*
Amino acid
Anti-HIV drugs
FLT
Glutamate
Nucleoside
NRTI
Organogel
Peptide
Reverse transcriptase

ABSTRACT

Designing microbicidal gels of anti-HIV drugs for local application to prevent HIV infection is a subject of major interest. 3′-Fluoro-3′-deoxythymidine (FLT), a nucleoside reverse transcriptase inhibitor (NRTI), was conjugated with a *N*-myristoylglutamate scaffold. The conjugate showed gelation at 1% (w/w) in different organic solvents, such as toluene, dichloromethane, and chloroform. The gels were opaque and stable at room temperature. The results indicate that myristoyl glutamate derivative of FLT can form an organogel. The gel could have potential application as a topical anti-HIV microbicidal agent.



Nucleoside analogues have been widely used for the treatment of different cancers[1–3] and human immunodeficiency virus (HIV) infection.[4] Nucleoside reverse transcriptase inhibitors (NRTIs) are the main class of anti-HIV drugs. Reverse transcriptase is an enzyme involved in RNA to DNA conversion in the HIV-infected cells.[5] NRTIs are used in combination with other drugs in highly active antiretroviral therapy (HAART).

HIV is mainly transmitted through sexual interactions. There is an urgent need to develop safe and effective preventative strategies.[6] Microbicides are topically applied agents that prevent or reduce transmission of infectious diseases, in particular HIV infection.[7] The topical microbicides are expected to be biocompatible, broad-spectrum, potent nontoxic, lacking detergent-type membrane toxicity, being harmless to the mucosal microflora, and to display broad-spectrum activity against drug-resistant HIV.

NRTIs are very polar compounds and may not be easily applicable as topical microbicides. Thus, it is important to convert the drug into a suitable form for topical applications. The gel form of drugs or drugs incorporated in gels[8] are one of the most convenient forms that have found application in diverse fields, including drug delivery.[9]

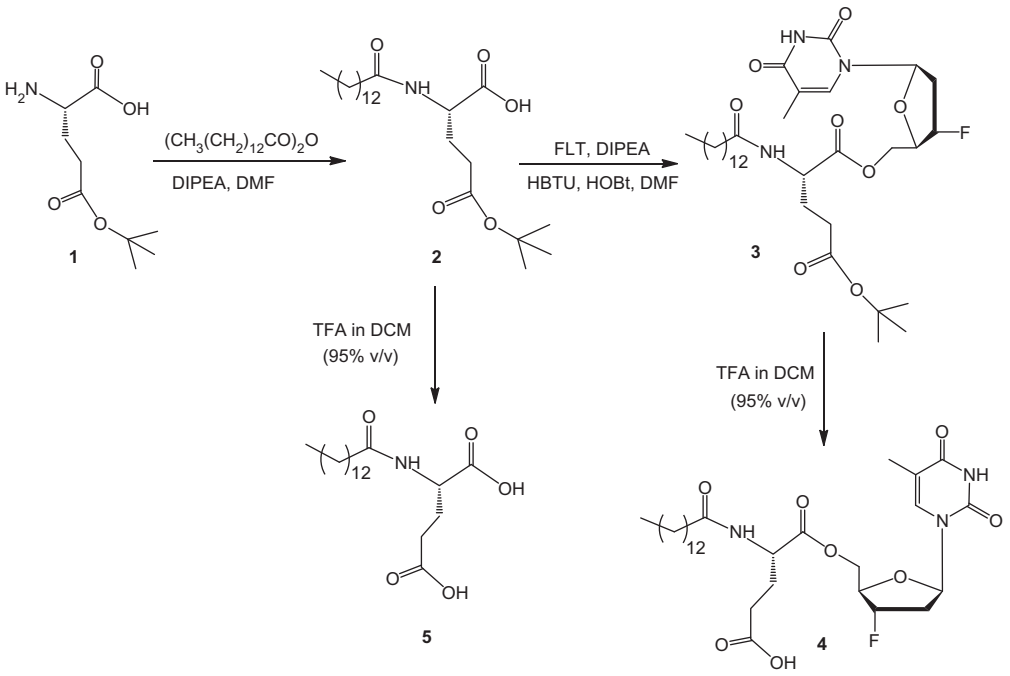
Organogels are semi-solid formulations with an organic liquid phase which is trapped by a three-dimensional network composed of self-assembled fibers. A number of organogelators have been reported for drug delivery application, such as lecithin, glyceryl fatty acid esters, poly(ethylene), *N*-lauroyl-glutamic acid di-*n*-butylamide, and *N*-stearoyl alanine methyl ester, mostly for dermal and transdermal formulations.[9] Herein, we report the gel formation by conjugation of a model nucleoside analogue, 3′-fluoro-2′,3′-dideoxythymidine (FLT) as a NRTI,[10] and a lipophilic myristoylated glutamic acid as organogelator. To the best of our knowledge, this is the first report of designing a gel by synthesizing lipophilic nucleoside-glutamic acid derivatives.

The glutamic acid–nucleoside conjugate derivative was synthesized starting from Glu(O*t*Bu)-OH (**1**) (Scheme 1). The myristoyl group was coupled to **1** by reaction with myristic anhydride in the presence of *N*,*N*-diisopropylethylamine (DIPEA) to yield myristoylated glutamic acid (*N*-My-Glu(O*t*Bu)-OH, **2**). Conjugation of **2** with FLT in the presence of 1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), DIPEA, and hydroxybenzotriazole (HOBt) afforded *N*-My-Glu(O*t*Bu)-OFLT (**3**). HOBt was used to protect the racemization of glutamic acid. Deprotection of *t*Bu in **3** was accomplished in the presence of TFA/DCM (95:5 v/v) to yield My-Glu(OH)-OFLT (**4**). The direct hydrolysis of myristoylated *t*-butylglutamic acid (**2**) with 95% TFA solution in DCM (v/v) gave *N*-myristoylglutamic acid (**5**, *N*-My-Glu-OH).

The gelation properties of the synthesized derivatives **4** and **5** were evaluated by dissolving them in different solvents at 1% w/w ratio, three times repeated treatment of heating the mixture to

* Corresponding author. Tel.: +1 401 874 4471; fax: +1 401 874 5787.
*E-mail addresses:* kparang@gmail.com, kparang@uri.edu (K. Parang).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.07.101




**Scheme 1.** Synthesis of *N*-myristoylglutamic acid derivative of FLT (**4**) and *N*-myristoylglutamic acid (**5**).

45 °C and sonication in a water bath sonicator for 5 min, followed by keeping the solution stable at room temperature for overnight.

The gelation of compounds **4** and **5** was evaluated in different solvents by a similar procedure and under similar conditions. As shown in Figure 1, *N*-myristoylated glutamate derivative of FLT **4** formed the white opaque gel in dichloromethane and toluene while the control *N*-myristoylated glutamic acid **5** did not form any gel under similar conditions. These data suggest that the presence of FLT is required for gel formation. The details of the gel formation in different solvents are shown in Table 1.

Furthermore, UV studies were conducted to find the solution to gel and gel to solution phase transition temperature in $CHCl_3$ solution. The cooling scan of the solution of compound **4** in chloroform from 50 to 5 °C followed by heating scan from 5 to 50 °C was monitored by change in UV absorbance at 265 nm (Fig. 2). The cooling scan graph on extrapolation of change on absorption pattern with temperature change gave the phase transition between 30 and 35 °C. The graph pattern was different during the heating scan and showed comparatively higher absorption or non-transmittance of light probably due to the presence of opaque gel form. There was a significant change in absorption graph from 35 to 40 °C, indicative of phase transition in this range. These data suggest the formation of low molecular weight organogel that is stabilized by weak inter-chain interactions. In non-aqueous conditions, the major attractive forces are possibly hydrogen bonding between glutamate amides, hydrogen bonding between nucleosides, and van der Waals interactions between alkyl chains of myristoyl groups. These data are consistent with previously reported gelation of single-walled carbon nanotubes functionalized with long fatty acyl chain,[11] suggesting the potential contribution of van der Waals interactions between alkyl chains for gel formation.

**Table 1**
Gel formation by compounds **4** and **5** in different solvents

| Compd | Solvent | Gel formation | Gel appearance |
|---|---|---|---|
| **4** | $CH_2Cl_2$ | Y[a] | Opaque, puff white |
| **4** | $CHCl_3$ | Y | Opaque, puff white |
| **5** | $CH_2Cl_2$ | N | Precipitate |
| **5** | Methanol | N | — |
| **4** | Methanol | N | — |
| **4** | Toluene | Y | Opaque, puff white |
| **4** | Xylene(s) | Y | Opaque, puff white |
| **5** | Toluene | N | — |
| **4** | Hexane | N | — |
| **5** | Hexane | N | — |
| **4** | Water (DMSO 1% v/v) | N[b] | Precipitate |
| **5** | Water | N | — |
| **4** | Ethanol | N | — |

[a] Y = gel formed.
[b] N = no gel.

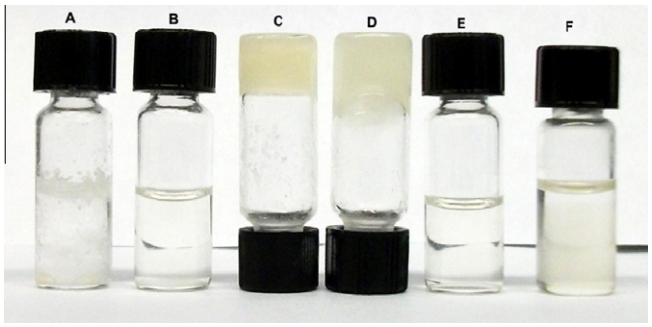



**Figure 1.** Gelation of compounds **4** and **5** in different solvents (1% w/w). (A) **4** in water (dissolved in DMSO and then added to water (water/DMSO 100:1 v/v); (B) **4** in methanol; (C) **4** in $CH_2Cl_2$; (D) **4** in toluene; (E) **5** in toluene; (F) **5** in $CH_2Cl_2$.

An organogel of **4** was generated by dissolving the gelator in toluene and heating. Upon cooling, the affinity between the gelator and organic solvent decreased leading to self-assembly into solid aggregates of gelator held through intermolecular interactions. Transmission electron microscopy (TEM) (Fig. 3) showed growth into fibers with 200–470 nanometers in width and up to several micrometers in length without breakage. Multidimensional growth pattern suggests a robust morphology.

A number of organogel formulations have been used in drug delivery. For example, lecithin has been used for the formulation

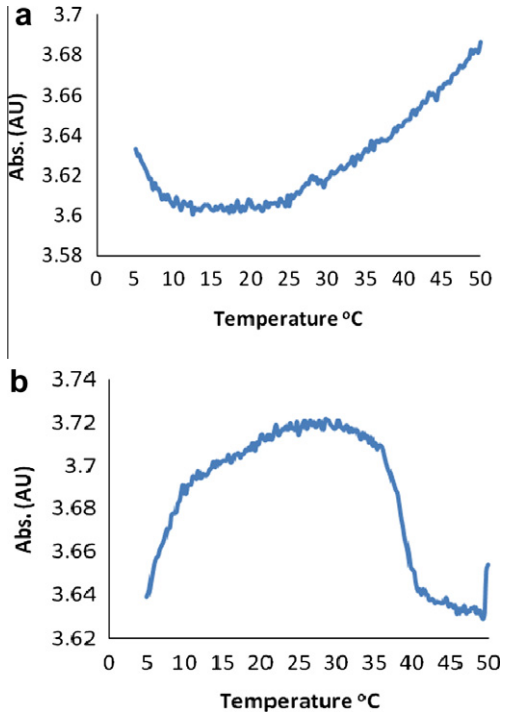



**Figure 2.** UV thermal graph for the compound **4** in $CHCl_3$ with absorbance at 265 nm. (a) Cooling graph from 50 to 5 °C; (b) heating graph from 5 to 50 °C. The reading is average of three readings for respective graph.

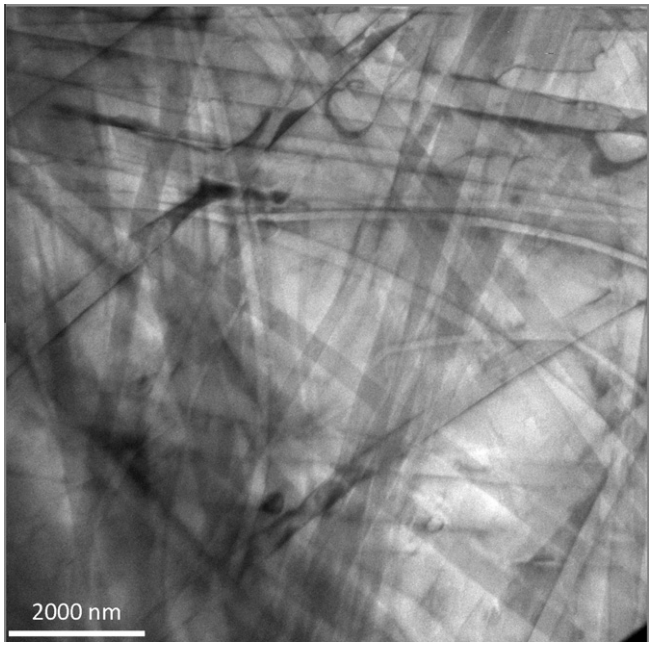



**Figure 3.** TEM of the organogel of **4** in toluene.

of diclofenac, piroxicam, and indomethacin.[7] Most of these formulations are used for transdermal, rectal, or subcutaneous administration because this strategy provides a non-invasive mode of administration minimizing the effects of organic solvents. Transdermal delivery offers net advantages over oral administration in terms of lowered systemic side effects associated with organic solvents. Gel components could be chosen according to their compatibility with intended applications, such as using non-toxic solvents for pharmaceutical applications. It is envisioned that organogel formation by **4** provides preliminary results for the potential use of nucleoside analogue organogels as topical anti-HIV microbicide applications. Optimization of the solvents to other nontoxic solvents and formulations are required to make these organogels appropriate for pharmaceutical applications. One major advantage of this organogel is that the active nucleoside pharmaceutical component is a part of gelator and does not need to be mixed with another drug, avoiding problems associated with the uncontrolled release of drugs from the formulation. FLT is expected to be released from the conjugate **4** through hydrolysis by cellular esterase as shown in other ester conjugates of nucleosides.[12]

In conclusion, the formation of organogels by FLT conjugation with *N*-myristoylglutamic acid was investigated. FLT-myristoyl glutamate conjugate formed organogel in dichloromethane, toluene, and xylene at 1% w/w. The formed gels were opaque and remained stable at room temperature. This method can be used for the gelation of other NRTIs conjugated with *N*-myristoylglutamic acid as the organogelator. This organogel NRTI can be used for the potential application as a topical anti-HIV microbicide. This strategy presents major advantages as drug delivery formulations, such as ease of preparation and route of administration.

## Acknowledgments

We thank the US National Science Foundation, Grant Number CHE 0748555 for the financial support and National Center for Research Resources, NIH, and Grant Number 1 P20 RR16457 for sponsoring the core facility.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.07.101.

## References and notes

1. Chhikara, B. S.; Parang, K. *Exp. Opin. Drug Del.* **2010**, *7*, 1399–1414.
2. Chhikara, B. S.; Mandal, D.; Parang, K. *Eur. J. Med. Chem.* **2010**, *45*, 4601–4608.
3. Kumar, S.; Mishra, A. K.; Chhikara, B. S.; Chuttani, K.; Sharma, R. K. *Hell. J. Nuclear Med.* **2008**, *11*, 91–95.
4. Agarwal, H. K.; Loethan, K.; Mandal, D.; Doncel, G. F.; Parang, K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1917–1921.
5. Costin, J. M. *Virol. J.* **2007**, *4*, 100.
6. Mehellou, Y.; De Clercq, E. *J. Med. Chem.* **2009**, *53*, 521–538.
7. Lederman, M. M.; Offord, R. E.; Hartley, O. *Nat. Rev. Immunol.* **2006**, *6*, 371–382.
8. Kang, L.; Liu, X. Y.; Sawant, P. D.; Ho, P. C.; Chan, Y. W.; Chan, S. Y. *J. Control. Rel.* **2005**, *106*, 88–98.
9. Vintiloiu, A.; Leroux, J.-C. *J. Control. Rel.* **2008**, *125*, 179–192.
10. Agarwal, H. K.; Chhikara, B. S.; Quiterio, M.; Doncel, G. F.; Parang, K. *J. Med. Chem.* **2012**, *55*, 2672–2687.
11. Pal, A.; Chhikara, B. S.; Govindaraj, A.; Bhattacharya, S.; Rao, C. N. R. *J. Mat. Chem.* **2008**, *18*, 2593–2600.
12. Agarwal, H. K.; Chhikara, B. S.; Hanley, M. J.; Ye, G.; Doncel, G. F.; Parang, K. *J. Med. Chem.* **2012**, *55*, 4861–4871.