Note

Journal of Labelled Compounds and Radiopharmaceuticals

Received 29 May 2009, Revised 29 July 2009, Accepted 14 September 2009 Published online 3 December 2009 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1689

A facile and efficient synthesis of d_6-labeled PU-H71, a purine-scaffold Hsp90 inhibitor

Tony Taldone, Danuta Zatorska, Yanlong Kang, and Gabriela Chiosis*

PU-H71 is a purine-scaffold Hsp90 inhibitor currently undergoing late stage preclinical evaluation for the treatment of cancer. In this investigation, we present a simple method for the synthesis of d_6-labeled PU-H71 for use as an internal standard to accurately quantitate the drug in biological matrices based on an LC-MS-MS method. PU-H71-d_6 was synthesized in five steps using readily available 1,3-dibromopropane-d_6 and is an important compound for the advancement of our clinical program.

Keywords: deuterium labeled PU-H71; purine; heat shock protein 90; inhibitor; cancer

Introduction

Molecular chaperone heat shock protein 90 (Hsp90) is essential for the proper folding and conformation of proteins and has recently emerged as an attractive target for the development of drugs against a variety of cancers.[1,2] Hsp90 has arisen to prominence as a cancer target because it plays a vitally important role in regulating clinically validated oncogenic proteins such as Her-2, ER and Bcr-Abl, as well as other signaling proteins that play a role in malignancy, such as Raf-1, p-Akt, Cdk4, mutant p53 and Flt-3.[3–6] Hsp90 functions as a chaperone through its ATPase activity and inhibition of this results in proteasomal degradation of its client proteins. The first synthetic small molecules to be discovered and translated into clinic in patients with advanced cancers are the purine-scaffold Hsp90 inhibitors,[1,2] and one such derivative, CNF-2024/BIIB021 (**1**), is currently in Phase I/II clinical trials for a variety of cancers (Figure 1). There are also non-purine synthetic Hsp90 inhibitors currently in clinical trials including the isoxazole VER-52296/NVP-AUY922 (**2**) and the 6,7-dihydro-indazol-4-one SNX-5422 (**3**). 17-AAG (**4**, tanespimycin) and IPI-504 (**5**, retaspimycin) are both derivatives of the natural product geldanamycin, and despite promising initial results for 17-AAG (**4**) clinical trials have recently been halted.

PU-H71 (**6**) is a second generation purine-scaffold Hsp90 inhibitor which our laboratory is currently developing for clinical trials. In support of our clinical program, we needed to develop a rapid and sensitive method for determining drug levels in biological matrices such as plasma and urine. One method evaluated for this purpose was LC-MS-MS utilizing an isotopically labeled analog, with identical physical and chromatographic properties to the unlabeled compound, as an internal standard. In this paper, we present the synthesis and characterization of PU-H71-d_6 (**7**).

Results and discussion

PU-H71 (**6**) can be obtained from a five-step synthetic sequence as shown in Scheme 1, commencing from 4,5,6-triaminopyrimidine sulfate (**8**). Cyclocondensation with CS_2 gives 8-mercaptoadenine (**9**) in quantitative yield and is used directly without further purification in the next step, to result in the 8-arylsulfanylpurine (**10**) in 60% yield via a copper catalyzed coupling to 1-iodo-3,4-methylenedioxybenzene. Reaction with N-iodosuccinimide gives **11** in 85% yield and alkylation with 1,3-dibromopropane results in **12** in 37% yield. PU-H71 (**6**) is obtained in the final step by reaction of **12** with excess isopropylamine in 90% yield.

Based on this synthetic sequence the simplest, most efficient and direct method for introducing the isotopic label would be during the final step using isopropylamine-d_7, which is readily available. Unfortunately, this moiety is not particularly stable as it is prone to fragment from the molecule on ionization in LC-MS-MS to yield identical product ions to the unlabeled compound **6**. The next logical step for the introduction of the label was in the 9-propyl chain in the penultimate step of the sequence and which remains intact during LC-MS-MS. The compound **11** was reacted with 1,3-dibromopropane-d_6 (**13**) to give **14** in 33% yield which was subsequently reacted with excess isopropylamine to give PU-H71-d_6 (**7**) in 92% yield (Scheme 2). The compound **7** was fully characterized by ^{1}H and ^{13}C NMR, HRMS and LCMS. The low yield obtained in the formation of **14** was expected from our previous experience with the unlabeled compound **12**, and can be primarily attributed to unavoidable competing 3-alkylation. Five equivalents of 1,3-dibromopropane is typically used in the preparation of **12** to ensure complete reaction of **11** and to limit other undesirable side-reactions such as dimerization. In an attempt to decrease the amount of 1,3-dibromopropane-d_6 (**13**) consumed we found that 3.7 equivalents can be used to obtain **14** in

Program in Molecular Pharmacology and Chemistry and Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

**Correspondence to: G. Chiosis, Program in Molecular Pharmacology and Chemistry and Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 482, New York, NY 10021, USA.*
E-mail: chiosisg@mskcc.org

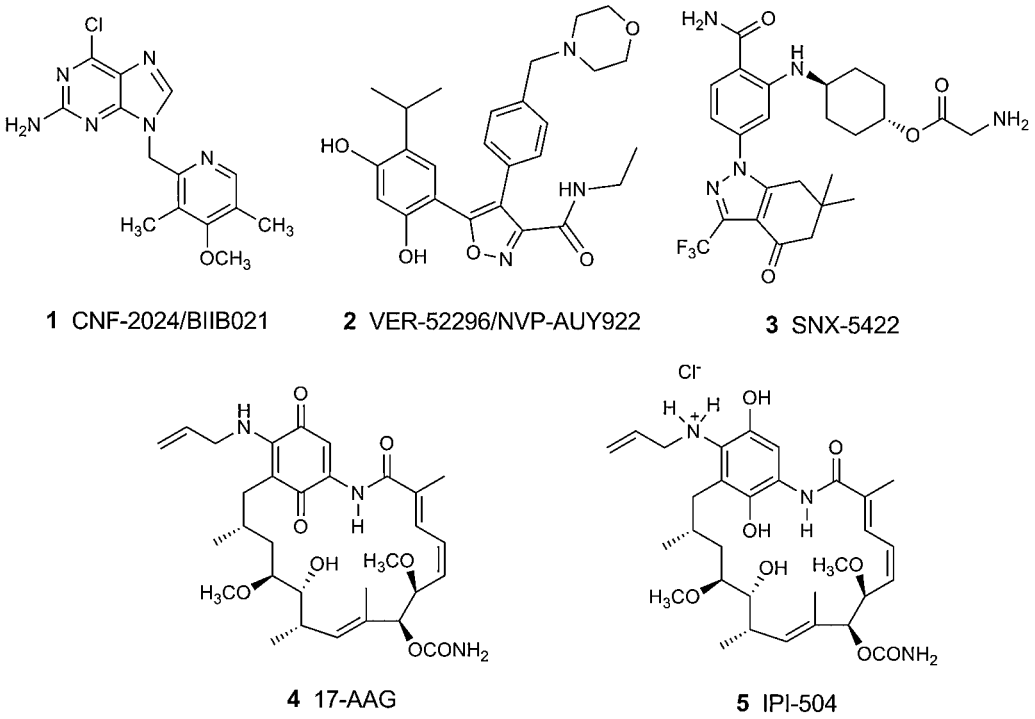
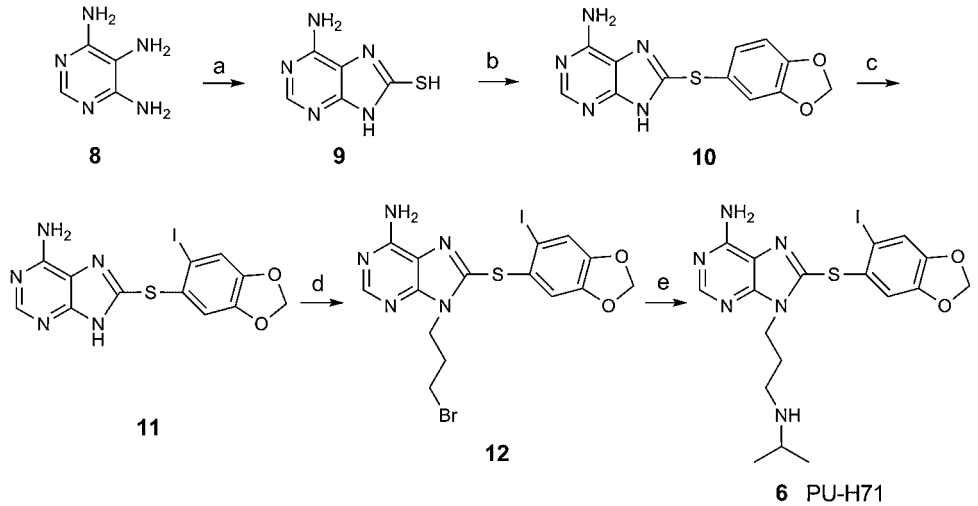


Figure 1. Some Hsp90 inhibitors that have been or are currently in clinical trials.



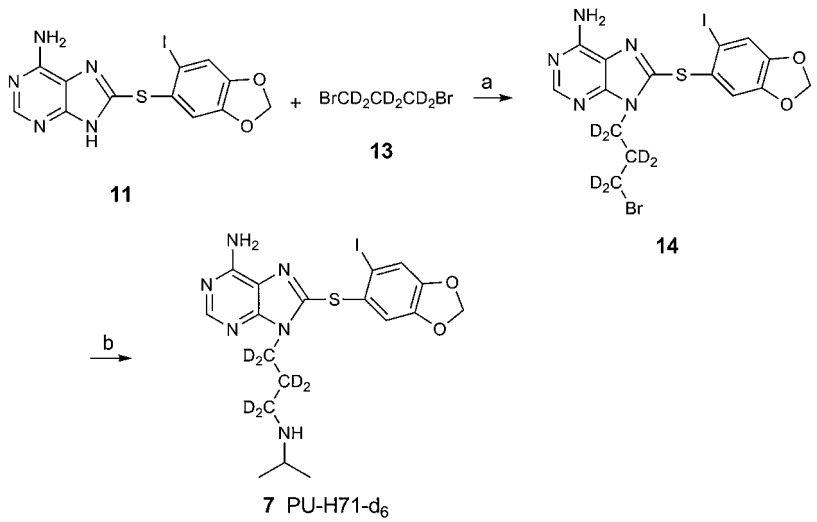
Reagents and conditions: (a) CS_2, $NaHCO_3$, H_2O, EtOH, reflux, 72 h, 100%; (b) 1-iodo-3,4-methylenedioxybenzene, neocuproine hydrate, CuI, NaOtBu, DMF, 115°C, 48 h, 60%; (c) NIS, CH_3CN, TFA, rt, overnight, 85%; (d) Cs_2CO_3, 1,3-dibromopropane, DMF, rt, 37%; (e) isopropylamine, DMF, rt, overnight, 90%.

Scheme 1. Synthesis of PU-H71 (**6**).

comparable yield. Further reducing the amount of **13** to three equivalents lowered the yield of **14** to 25%.

Experimental

11 was synthesized as previously reported.[7] 1,3-dibromopropane-d_6 (**13**) was purchased from Aldrich and certified to be 99.7 atom% D. ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz instrument. Chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard. ^1H data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet, q, quartet, br, broad, m, multiplet), coupling constant (Hz), integration. ^{13}C chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard. High-resolution mass spectra were recorded on a Waters LCT Premier system. Low-resolution mass spectra were obtained on Waters Acquity Ultra Performance LC with electrospray ionization and SQ detector. High-performance liquid chromatography analyses were performed on a Waters Autopurification system with PDA, MicroMass ZQ and ELSD



Scheme 2. Synthesis of PU-H71-d_6 (**7**).

detector and a reversed phase column (Waters X-Bridge C18, 4.6 × 150 mm, 5 μm) using a gradient of (a) H_2O+0.1% TFA and (b) CH_3CN+0.1% TFA, 5–90% b over 10 min. Column chromatography was performed using 230–400 mesh silica gel (EMD). All reactions were performed under argon protection.

9-(3-bromopropyl-d_6)-8-(6-iodobenzo[d][1,3]dioxol-5-ylthio)-9H-purin-6-amine (14)

In 5.5 mL of DMF 0.188 g (0.455 mmol) of **11** was dissolved. A total of 0.165 g (0.505 mmol) of Cs_2CO_3 and 0.350 g (171 μL, 1.68 mmol) of 1,3-dibromopropane-d_6 (**13**) were added and the mixture was stirred at rt for 45 min. Then additional Cs_2CO_3 (0.027 g, 0.083 mmol) was added and the mixture was stirred for 45 min. Solvent was removed under reduced pressure and the resulting residue was chromatographed (CH_2Cl_2:MeOH:AcOH, 120:1:0.5 to 80:1:0.5) to give 0.082 g (33%) of **14** as a white solid. ^{1}H NMR ($CDCl_3$/CD_3OD) δ 8.25 (s, 1H), 7.38 (s, 1H), 7.03 (s, 1H), 6.05 (s, 2H); MS (ESI) *m/z* 539.7/541.8 $[M+H]^+$.

PU-H71-d_6 (7)

0.082 g (0.152 mmol) of **14** and 0.449 g (0.647 mL, 7.6 mmol) of isopropylamine were dissolved in 2 mL of DMF and stirred at rt for 14 h. Solvent was removed under reduced pressure and the resulting residue was chromatographed (CH_2Cl_2:MeOH:MeOH-NH_3 (7 N), 90:0.5:0.5 to 30:0:1) to give 0.072 g (92%) of **7** as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H), 7.27 (s, 1H), 6.86 (s, 1H), 6.30 (br s, 2H), 5.95 (s, 2H), 2.70 (septet, J = 6.3 Hz, 1H), 1.02 (d, J = 6.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 154.8, 153.0, 151.6, 149.2, 148.9, 146.0, 128.1, 120.2, 119.2, 112.1, 102.3, 90.9, 48.6, 23.0; MS (ESI) *m/z* 518.9 $[M+H]^+$; HPLC: R_t = 6.37 min.; HRMS (ESI) *m/z* $[M+H]^+$ calcd. for $C_{18}H_{16}D_6IN_6O_2S$, 519.0946; found 519.0965.

Conclusion

We have prepared PU-H71-d_6 (**7**) as a valuable internal standard for an LC-MS-MS method designed to quantify PU-H71 concentration in biological samples from preclinical studies and eventually from subjects in clinical studies. The compound **7** was synthesized from commercially available 4,5,6-triaminopyrimidine sulfate (**8**) in five steps overall with the introduction of the label in the penultimate step via 1,3-dibromopropane-d_6 (**13**). Therefore, the described synthesis represents a facile and efficient route to d_6-labeled PU-H71, a critically important compound for the advancement of our clinical program.

Acknowledgement

This work was supported in part by W. H. Goodwin and A. Goodwin and the Commonwealth Cancer Foundation for Research, The Experimental Therapeutics Center of Memorial Sloan-Kettering Cancer Center (MSKCC), the Translational and Integrative Medicine Research Fund of MSKCC, the Byrne Fund and the Geoffrey Beene Cancer Research Center of MSKCC (G. C.).

References

[1] T. Taldone, W. Sun, G. Chiosis, *Bioorg. Med. Chem.* **2009**, *17*, 2225–2235.
[2] T. Taldone, A. Gozman, R. Maharaj, G. Chiosis, *Curr. Opin. Pharmacol.* **2008**, *8*, 370–374.
[3] A. Kamal, M. F. Boehm, F. J. Burrows, *Trends Mol. Med.* **2004**, *10*, 283–290.
[4] L. Neckers, K. Neckers, *Expert Opin. Emerg. Drugs* **2005**, *10*, 137–149.
[5] M. V. Powers, P. Workman, *Endocr. Relat. Cancer* **2006**, (Suppl 1), S125–S135.
[6] G. Chiosis, *Expert Opin. Ther. Targets* **2006**, 10, 37–50.
[7] H. He, D. Zatorska, J. Kim, J. Aguirre, L. Llauger, Y. She, N. Wu, R. M. Immormino, D. T. Gewirth, G. Chiosis, *J. Med. Chem.* **2006**, *49*, 381–390.