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Arylethynyltriazole acyclonucleosides inhibit hepatitis C virus replication

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Abstract—Novel acyclic triazole nucleosides with various ethynyl moieties appended on the triazole nucleobase were synthesized efficiently using a convenient one-step Sonogashira reaction in aqueous solution and under microwave irradiation. One of the compounds, **1f**, inhibited HCV subgenomic replication with a 50% effective concentration (EC_{50}) of 22 μg/ml and did not inhibit proliferation of the host cell at a concentration of 50 μg/ml. A preliminary SAR study suggests that the appended phenyl ring as well as the rigid triple bond linker contributes importantly to the anti-HCV activity.

Hepatitis C virus (HCV) infection constitutes a serious public health problem: an estimated 3% of the world population—about 170 millions people—are infected by HCV.[1] Current standard for the treatment of HCV is pegylated interferon-α in combination with ribavirin.[2] However, this therapy is only effective in about 50% of the patients and is associated with serious side effects.[2] Therefore, there is an urgent need to develop more efficacious and better tolerated new antiviral candidates for combating HCV.[3]

Of the drugs currently marketed for the treatment of viral infection, almost half of them are nucleoside analogs. Nucleoside analogs with modified sugar and/or base components can mimic natural nucleosides and serve as building units or inhibitors to interfere in the nucleic acid synthesis or to block the biological processes involving the action of nucleos(t)ides. Ribavirin (Scheme 1), the nucleobase of which consists of an unnatural triazole moiety, was the first synthetic nucleoside to show a broad spectrum of an antiviral activity against many RNA and DNA viruses.[4] It is the only small molecular drug available to date for treating patients infected with hepatitis C virus.[2] Recently, nucleoside analogs with either a 2′-C-methyl, a 2′-F or 4′-azido modification of the sugar moiety were identified as inhibitors of HCV replication; the 5′-triphosphate metabolites of these drugs inhibit the viral polymerase.[5]

Appending aromatic systems to nucleobase may yield nucleosides with unique properties and biological activities since the appended aromatic systems may promote advantageous binding properties to the corresponding nucleosides for interaction with biological targets through stronger and more efficient binding via larger aromatic systems. Successful examples are 6-arylpurine nucleosides (Scheme 1), which have been shown to elicit anti-HCV and anticancer activities.[6] HEPT, an acyclonucleoside analog of which the pyrimidine nucleobase bears a phenylthio group at the 6-position (Scheme 1), is another example that has been reported to show a potent and selective anti-HIV activity.[7] The appended aryl at the 6-position in HEPT is one of the key factors contributing to the anti-HIV activity. X-ray structural analysis revealed that HEPT acts as a non-nucleoside inhibitor of the HIV reverse transcriptase and that the aryl group at the 6-position in HEPT interacts with the amino acid residues Tyr188 and Leu100 in the HIV reverse transcriptase, leading to the conformational change of this enzyme.[8]

In our ongoing efforts to search for potent new triazole nucleosides with antiviral activity,[9–11] we are interested in developing acyclic triazole nucleosides with π-conjugated aromatic systems appended on the triazole ring.

Keywords: Triazole nucleosides; Antiviral activity; Anti-HCV; Sonogashira reaction; Microwave-irradiation promoted reaction.
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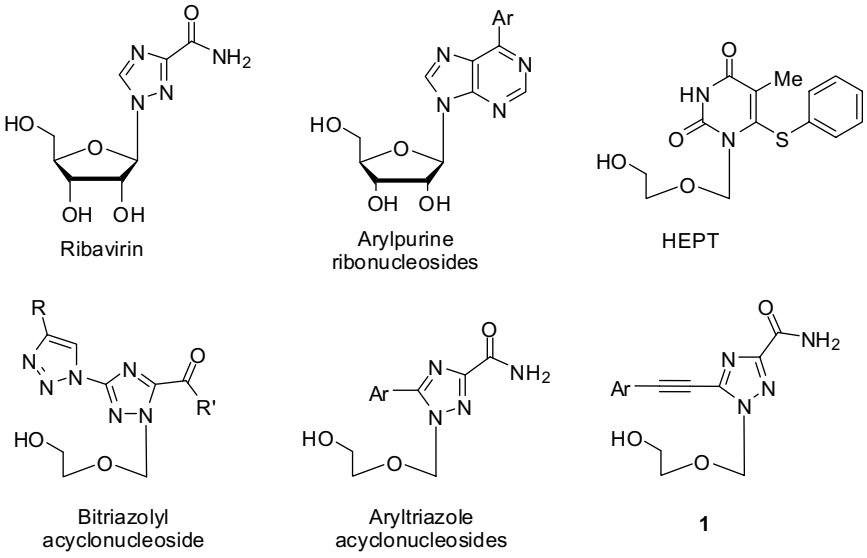


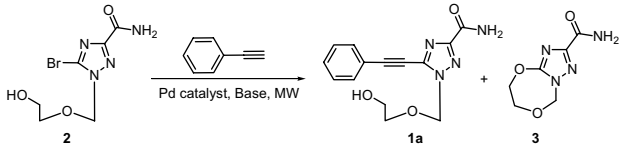
Scheme 1. Ribavirin, 6-arylpurine ribonucleosides, HEPT and various aryltriazole acyclonucleoside derivatives.

We expect that these appended aromatic systems will offer the triazole advantageous binding properties to the corresponding biological targets via larger aromatic systems. Furthermore, nucleosides with unnatural triazole nucleobases are generally resistant to nucleos(t)ide metabolizing enzymes, and may lead to a better in vivo stability and efficiency. Until now, very few efforts have been made on appending aromatic systems to triazole nucleosides, probably due to the lack of convenient and practical synthetic methods. Making use of Huisgen cycloaddition and Suzuki coupling reaction, we have recently synthesized bitriazolyl[10] and aryltriazole acyclonucleosides[11] (Scheme 1), some of which displayed interesting antiviral activity against the tobacco mosaic virus.[10] Here, we report the synthesis of a new family of triazole nucleosides, 5-arylethynyltriazole acyclonucleosides **1** (Scheme 1), and the evaluation of their antiviral activity against HCV.

Sonogashira reaction[12] is one of the most straightforward and convenient approach to introduce an alkynyl functional group on aromatic systems and heterocyclic nucleobases. Starting from bromotriazole acyclonucleoside (**2**),[11a] we developed herein a simple and efficient one-step procedure to synthesize ethynyltriazole acyclonucleosides (**1**) in an aqueous solution via a Sonogashira reaction promoted by microwave irradiation (Tables 1 and 2).

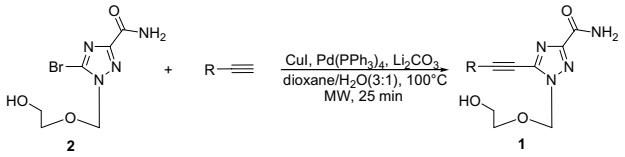
Table 1 summarized our efforts to optimize the experimental conditions for such Sonogashira reaction between the bromotriazole derivative **2** and phenylacetylene. We know from our previous experience that the starting material **2** undergoes easily intra-molecular cyclization under basic conditions, leading to the formation of **3**.[11a] Our optimization effort was therefore

Table 1. Optimization of Sonogashira reaction under microwave irradiation

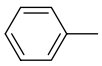
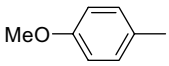
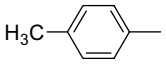
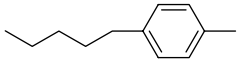
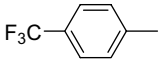
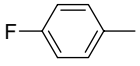
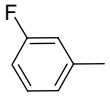
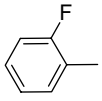
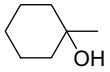
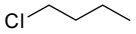
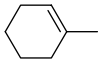
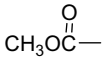
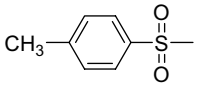


Entry	Solvent	Base (2 equiv)	Pd catalyst (5%)	Temperature (°C)	**1a** (%)	**3** (%)	**2** (%)
1	H_2O	Li_2CO_3	$Pd(PPh_3)_4$:CuI (1:1)	100	0	30	0
2	Dioxane/H_2O (1:1)	Li_2CO_3	$Pd(PPh_3)_4$:CuI (1:1)	100	36	0	60
3	Dioxane/H_2O (3:1)	Li_2CO_3	$Pd(PPh_3)_4$:CuI (1:1)	100	99	0	0
4	CH_3CN/H_2O (3:1)	Li_2CO_3	$Pd(PPh_3)_4$:CuI (1:1)	100	77	0	10
5	THF/H_2O (3:1)	Li_2CO_3	$Pd(PPh_3)_4$:CuI (1:1)	100	88	0	0
6	Dioxane/H_2O (3:1)	Li_2CO_3	$Pd(PPh_3)_2Cl_2$:CuI (1:1)	100	76	0	0
7	Dioxane/H_2O (3:1)	Li_2CO_3	$Pd_2(dba)_3$:CuI (1:1)	100	0	0	83
8	Dioxane/H_2O (3:1)	Li_2CO_3	$Pd(OAc)_2$:CuI: ± BINAP (1:1:2)	100	5	0	66
9	Dioxane/H_2O (3:1)	Li_2CO_3	$Pd(PPh_3)_4$	100	30	0	60
10	Dioxane/H_2O (3:1)	Li_2CO_3	2.5% $Pd(PPh_3)_4$:CuI (1:1)	100	49	0	50
11	Dioxane/H_2O (3:1)	K_2CO_3	$Pd(PPh_3)_4$:CuI (1:1)	100	83	9	0
12	Dioxane/H_2O (3:1)	Et_3N	$Pd(PPh_3)_4$:CuI (1:1)	100	93	0	0
13	Dioxane/H_2O (3:1)	Li_2CO_3	$Pd(PPh_3)_4$:CuI (1:1)	80	65	0	32

Table 2. Synthesis of **1** via the Sonogashira reaction between **2** and various terminal alkynes



2 **1**

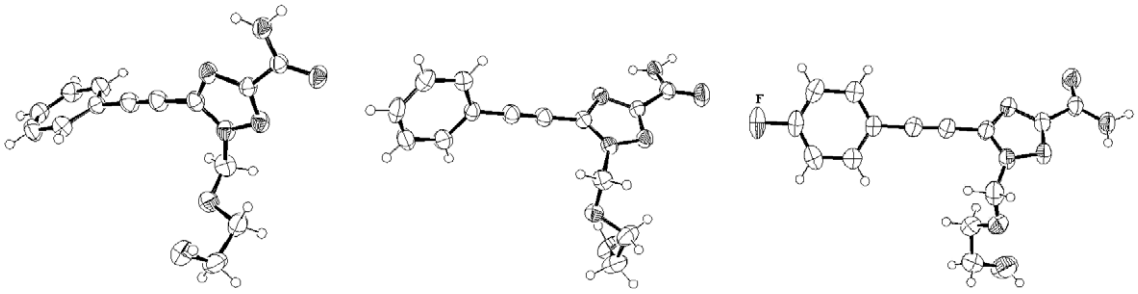
Entry	R	Product	Yield[a] (%)
1		**1a**	99
2		**1b**	91
3		**1c**	90
4		**1d**	92
5		**1e**	75
6		**1f**	86
7		**1g**	77
8		**1h**	85
9		**1i**	97
10		**1j**	91
11		**1k**	93
12		**1l**	90
13		**1m**	0
14		**1n**	0

[a] 0.05 equiv $Pd(PPh_3)_4$, 0.05 equiv CuI, 2.0 equiv Li_2CO_3, dioxane/H_2O (3:1), 100 °C, microwave irradiation, 25 min.

mainly focused on preventing this cyclization side reaction while promoting Sonogashira reaction. We first attempted to use water as the solvent because of its ecologic and economic advantages as well as the fact that the starting material **2** can be well solubilized in water. Unfortunately, the reaction in pure water did not yield the desired product, but only the cyclization by-product **3** (Table 1, entry 1). Using mixed solvent systems could efficiently suppress this side reaction (Table 1, entries 2–5). The best result was obtained with dioxane/H_2O (3:1, v/v) which gave the desired product **1a** in almost quantitative yield (Table 1, entry 3). Among the four common Pd-catalysts tested, $Pd(PPh_3)_4$, $Pd(PPh_3)_2Cl_2$, $Pd_2(dba)_3$, and $Pd(OAc)_2$ (Table 1, entries 3, 6, 7, and 8), $Pd(PPh_3)_4$ was identified as the most efficient. Furthermore, CuI is an important co-catalyst since its absence led to the incomplete transformation of the starting material **2** and significantly lower yield of the desired product **1a** (Table 1, entry 9). It is also important to maintain the catalyst loading to 5% because lower catalyst loading led to a dramatic yield decrease (Table 1, entry 10). In order to avoid the formation of the cyclization by-product **3** under the strong basic conditions, we employed relatively weak bases such as K_2CO_3, Li_2CO_3, and triethylamine. All the three bases offered good to excellent yields (Table 1, entries 3, 11, and 12). However, the use of triethylamine was not recommended since the triethylammonium salt formed during the reaction disturbed the purification of the desired product by chromatography on silica gel. We have therefore chosen Li_2CO_3 because of its excellent performance. Finally, we found out that the optimal reaction temperature was 100 °C. Indeed, higher temperatures led to the decomposition of the palladium catalyst (data not shown), while lower temperature could not ensure efficient transformation (Table 1, entry 13). The reaction time was also finely tuned to 25 min in order to achieve maximum yields, since this enabled the complete reaction to occur, while preventing the decomposition of the products (data not shown).

Under our optimized conditions, that is, with $Pd(PPh_3)_4$/CuI and Li_2CO_3, in dioxane/H_2O (3:1, v/v) as solvents, we were able to obtain the 5-arylethynyl and 5-alkylethynyl analogs of triazole acyclonucleosides[13] with good to excellent yields when the alkynes were not directly linked with the electron-withdrawing groups (Table 2, entries 1–4 and 9–12). The presence of electron-withdrawing groups such as F or CF_3 on the phenylacetylene resulted in slightly lower reaction yields (Table 2, entries 5–8), whereas alkynes directly linked to the electron-withdrawing functionalities completely blocked the Sonogashira reaction and no product could be identified (Table 2, entries 13 and 14). Two factors may be responsible of these results. The first one is that strong electron-withdrawing group will reduce significantly the nucleophilicity of the corresponding alkyne, making the transmetalation step difficult to occur. The second one is that the strong electron-withdrawing group will increase electrophilicity of the corresponding alkyne, leading to undesired Michael additions.[14,15] It is therefore a synthetic challenge to introduce electrophilic alkynyl functionalities on a triazole ring with high yields by using Sonogashira reaction.

We further obtained crystals of **1a** and **1f** and their X-ray structures.[16,17] It is worth to mention that two different conformations, (I) and (II) (Fig. 1), exist for **1a** in its crystal structures, which probably results from H-bonding and crystal packing. In both compounds **1a** and **1f**,



1a (I) **1a** (II) **1f**

Figure 1. X-ray structures of compounds **1a** and **1f**.

the phenyl ring extended out of the triazole ring plane via the triple bond linkage (Fig. 1), which differ from those previously observed for the bitriazolyl[10] and aryltriazolyl nucleosides.[11]

Antiviral activity of the above synthesized triazole nucleosides (Table 3) was first evaluated in a HCV subgenomic RNA replicon assay using Huh-5-2 cells.[18,19] All the compounds with *arylethynyl* functionalities on the triazole ring **1a–1h**, except **1b**, exhibited anti-HCV activity (Table 3, entries 1–8), whereas compounds bearing an *alkylethynyl* substituent **1i–1l** were devoid of anti-HCV activity (entries 9–12 in Table 3). This may suggest that the appended aromatic systems brought by the arylethynyl functionality on the triazole ring in **1a–1h** are responsible for the antiviral activity, which is in line with our initial concept for molecular design.

Compounds **1a** and **1f** inhibited HCV replication, without being cytotoxic (Table 3, entries 1 and 6).[20] Preliminary delineation on the anti-HCV activity and the toxicity of compounds **1a–f** could be obtained, based on the different functionalities at the *p*-position of the phenylacetylene moiety (Table 3, entries 1–6). Compound **1b** exhibited no anti-HCV activity (Table 3, entry 2), which may be due to the unfavorable electronic properties brought by the methoxyl group on the phenyl ring. Cytotoxicity was observed with compounds **1d**, **1e**, and **1g** (Table 3, entries 4, 5, and 7). Although both **1c** and **1h** had a CC_{50} value over 50 μg/mL, these inhibited at 50 μg/mL host cell proliferation (data not shown). Overall, the cytotoxicity might be the consequence of the steric congestion brought by the large and/or lipophilic substituents such as $–C_6H_{13}$, $–CH_3$, $–CF_3$, and F.

The anti-HCV activity of **1a** and **1f** was further assessed in two other HCV subgenomic RNA replicon assays using Huh-9-13 and Huh-6 cells.[18] Compound **1a** had an EC_{50} value over 50 μg/mL in Huh-9-13 cells, while the activity of **1f** as observed in Huh-5-2 cells was corraborated by the activity noted in Huh-9-13 and Huh-6 cells with EC_{50} values of 38 ± 2 and 29 ± 1 μg/mL, respectively. In addition, **1f** is superior to ribavirin in selectivity, because it is much less toxic[20] than ribavirin although it is slightly less potent.

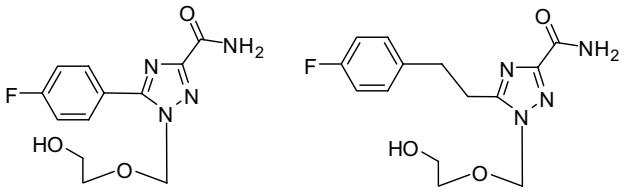
In view of these results, we further studied the importance of the triple bond bridging the phenyl ring and the triazole ring in **1f**. Removing this rigid ethynyl triple bond in **1f** led to the inactive analog **4**[11a] (Scheme 2), whereas replacing the rigid triple bond in **1f** by the more flexible ethylene group in **5**[21] (Scheme 2) abolished completely the antiviral activity (data not shown). Therefore, the triple bond linker, between the phenyl and triazole rings, seems to be also critical for the antiviral activity observed for **1f**.

Finally, we compared the crystal structure of **1f** with that of HEPT.[7a] We noticed that the structure of **1f** resembles closely to that of HEPT (Fig. 2). HEPT is reported to be a non-nucleoside inhibitor of the reverse transcriptase of HIV, which binds to the allosteric site and exerts its antiviral effect by inducing conformational changes of the reverse transcriptase of HIV.[8] Although the close structural resemblance of **1f** to HEPT may lead us to speculate that **1f** may target the RNA-dependent-RNA-polymerase of HCV, it is worth to note that the crystal structure may not necessarily represent the biologically active form.

Table 3. Antiviral activities of synthesized compounds on HCV Subgenomic replicon replication in Huh-5-2 Cells[a]

Entry	Compound	EC_{50}[b] (μg/mL)	CC_{50}[c] (μg/mL)
1	**1a**	18 ± 4[d]	>50
2	**1b**	>50	>50
3	**1c**	16	>50, T[e]
4	**1d**	22	25
5	**1e**	5	20
6	**1f**	22 ± 3[d]	>50
7	**1g**	10	29
8	**1h**	20	>50, T[e]
9	**1i**	>50	>50
10	**1j**	>50	>50
11	**1k**	>50	>50
12	**1l**	>50	>50
13	Ribavirin	7 ± 2	21 ± 11

[a] Interferon-α-2b at 10,000 U/well reduced the signal in the viral RNA (luciferase) assay to background levels; without any cytostatic activity.
[b] Effective concentration (EC_{50}): concentration required to inhibit luciferase activity in the replicon system by 50%.
[c] Cytotoxic concentration (CC_{50}): concentration required to inhibit the proliferation of exponentially growing Huh-5-2 cells by 50%.
[d] Average values based on six independent assays.
[e] T: $CC_{50} > 50$ μg/mL, however, toxicity (cell growth <80%) was observed at sample concentration of 50 μg/mL.



Scheme 2. Compounds **4** and **5** as structural analogs of **1f**.

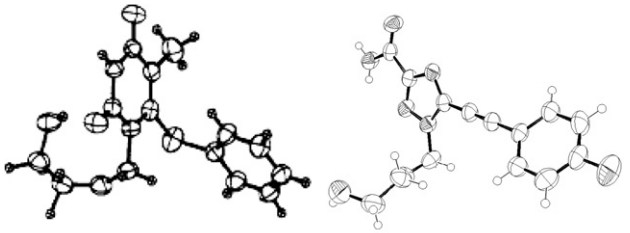


Figure 2. Crystal structures of HEPT[7a] and **1f**.

In conclusion, we synthesized a series of novel arylethynyltriazole acyclonucleosides using a simple and convenient one-step Sonogashira coupling reaction in aqueous solution and promoted by microwave irradiation. One of the compound **1f** selectively inhibited HCV replication. Preliminary structure/activity relationship analysis revealed that the appended aromatic phenyl ring, the substituents at the *p*-position of the phenyl ring as well as the rigid triple bond connection between the phenyl group and the triazole ring contribute critically to the anti-HCV activity. Analogs with large and lipophilic substituents on the ethynylphenyl ring may have a more pronounced inhibitory effect on proliferation of the host cell. The arylethynyltriazole acyclonucleosides disclosed here present simple and concise structural motifs, which can be conveniently synthesized using the Sonogashira reaction and easily modified for further structural optimization and structural/activity relationship study. These constitute therefore an interesting structural lead in the search for novel antiviral compounds against HCV, for which there is no efficient treatments and development of new and efficacious antiviral agents is of extreme importance and urgence.

Acknowledgments

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Supplementary data

NMR spectra of all the new compounds are included. This material is available free of charge via the Internet. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.026.

References and notes

1. Weiss, U., Eds., *Nature* **2005**, *436*, 929.
2. Feld, J. J.; Hoofnagle, J. H. *Nature* **2005**, *436*, 967.
3. (a) De Francesco, R.; Migliaccio, G. *Nature* **2005**, *436*, 953; (b) Gordon, C. P.; Keller, P. A. *J. Med. Chem.* **2005**, *48*, 1.
4. Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. *Science* **1972**, *177*, 705.
5. Carroll, S. S.; Olsen, D. B. *Infect. Disord. Drug Targets* **2006**, *6*, 17.
6. (a) Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817; (b) Hocek, M.; Nauš, P.; Pohl, R.; Votruba, I.; Furman, P. A.; Tharnish, P. M.; Otto, M. J. *J. Med. Chem.* **2005**, *48*, 5869.
7. (a) Miyasaka, T.; Tanaka, H.; Baba, M.; Hayakawa, H.; Walker, R. T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 2507; (b) Tanaka, H.; Baba, M.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Shigeta, S.; Walker, R. T.; De Clercq, E.; Miyasaka, T. *J. Med. Chem.* **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. *J. Med. Chem.* **1991**, *34*, 1508.
8. Ren, J. S.; Esnouf, R.; Garman, E.; Somers, D.; Ross, C.; Kirby, I.; Keeling, J.; Darby, G.; Jones, Y.; Stuart, D.; Stammers, D. *Nat. Struct. Biol.* **1995**, *2*, 293.
9. Wu, Q. Y.; Qu, F. Q.; Wan, J. Q.; Zhu, X.; Xia, Y.; Peng, L. *Helv. Chim. Acta* **2004**, *87*, 811.
10. (a) Li, W.; Xia, Y.; Fan, Z. J.; Qu, F. Q.; Wu, Q. Y.; Peng, L. *Tetrahedron Lett.* **2008**, *49*, 2804; (b) Xia, Y.; Li, W.; Qu, F. Q.; Fan, Z. J.; Liu, X. F.; Berro, C.; Rauzy, E.; Peng, L. *Org. Biomol. Chem.* **2007**, *5*, 1695; (c) Xia, Y.; Fan, Z. J.; Yao, J. H.; Liao, Q.; Li, W.; Qu, F. Q.; Peng, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2693; (d) Xia, Y.; Qu, F. Q.; Li, W.; Wu, Q. Y.; Peng, L. *Heterocycles* **2005**, *65*, 345.
11. (a) Zhu, R. Z.; Qu, F. Q.; Quéléver, G.; Peng, L. *Tetrahedron Lett.* **2007**, *48*, 2389; (b) Wan, J. Q.; Zhu, R. Z.; Xia, Y.; Qu, F. Q.; Wu, Q. Y.; Yang, G. F.; Neyts, J.; Peng, L. *Tetrahedron Lett.* **2006**, *47*, 6727.
12. (a) Chinchilla, R.; Nájera, C. *Chem. Rev.* **2007**, *107*, 874; (b) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875; (c) Hocek, M. *Eur. J. Org. Chem.* **2003**, 245.
13. *General:* All the terminal alkynes and catalysts were purchased from Acros or Lancaster. The microwave assisted reactions were performed on an Initiator™ Creator produced by Biotage. The ^{1}H NMR spectra were recorded at 300 or 600 MHz and the ^{13}C NMR spectra were recorded at 75 or 150 MHz, respectively, on Varian Mercury-VX300 and Varian Inova-600 spectrometers. The chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. FAB and ESI mass spectra were determined using ZAB-HF-3F and Finnigan LCQ Advantage mass spectrometers, respectively. High resolution mass spectra were obtained by Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) using an IonSpec 4.7 T Fourier Transform Mass Spectrometer. All the compounds were purified by performing flash chromatography on silica gel (200–300 mesh).
Procedure for preparing **1** *via a microwave assisted Sonogashira reaction:* The terminal alkynes (0.24 mmol), tetrakis(triphenylphosphine)palladium(0) (11.6 mg, 0.01 mmol),

CuI (2.2 mg, 0.01 mmol), Li_2CO_3 (30.5 mg, 0.4 mmol) and 5-bromo-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide **2** (0.2 mmol) were suspended in 2.8 mL of dioxane/H_2O (3/1) under argon. The vessel was sealed and irradiated at 100 °C for 25 min, and then cooled to room temperature. The reaction mixture was concentrated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (CH_2Cl_2/CH_3OH, 20:1). The purified material was dried in vacuo to afford the corresponding derivative derivatives **1a–1l**.

5-Phenylethynyl- 1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1a**):White solid (99 %).^{1}H NMR (300 MHz, DMSO-d_6): δ 8.06 (br s, 1H, –C(O)N*H*), 7.73–7.75 (m, 3H, –C(O)N *H* and Ar*H*), 7.50–7.58 (m, 3H, Ar*H*), 5.75 (s, 2H, *H*-1′), 4.76 (br s, 1H, –O*H*), 3.63 (t, *J* = 5.1 Hz, 2H, *H*-2′), 3.51–3.53 (m, 2H, *H*-3′); ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.4, 157.6, 140.6, 132.8, 131.5, 129.8, 120.1, 97.6, 78.8, 75.6, 72.1, 60.5; FAB-MS: *m/z* 287 $[M+H]^+$, 309 $[M+Na]^+$; HRMS: Calcd for $C_{14}H_{15}N_4O_3^+$ 287.1139. Found 287.1148. IR: 2232.70 cm^{-1} (–C≡C–).

5-[(4-Methoxyphenyl)ethynyl]-1-[(2-hydroxyethoxy)-methyl]-1,2,4-triazole-3-carboxamide (**1b**):White solid (91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.03 (br s, 1H, –C(O)N *H*), 7.74 (br s, 1H, –C(O)N *H*), 7.68 (d,*J* = 8.9 Hz, 2H, Ar*H*), 7.07 (d, *J* = 8.9 Hz, 2H, Ar*H*), 5.72 (s, 2H, *H*-1′), 4.76 (t, *J* = 5.6 Hz, –O *H*), 3.83 (s, 3H, –OCH_3), 3.62 (t, *J* = 4.8 Hz, 2H, *H*-2′), 3.49–3.54 (m, 2H, *H*-3′); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.8, 160.5, 157.5, 141.0, 134.6, 115.5, 111.8, 98.2, 78.7, 74.7, 72.1, 60.5, 56.2; FAB-MS: *m/z* 317 $[M+H]^+$, 339 $[M+Na]^+$; HRMS: Calcd for $C_{15}H_{17}N_4O_4^+$ 317.1244. Found 317.1248; IR: 2217.69 cm^{-1} (–C≡C–).

5-[(p-Tolyl)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1c**)*:* White solid (90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.06 (br s, 1H, –C(O)N*H*), 7.76 (br s, 1H, –C(O)N*H*), 7.63 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.34 (d, *J* = 8.0 Hz, 2H, Ar*H*), 5.73 (s, 2H, *H*-1′), 4.77 (t, *J* = 5.4 Hz, –O*H*), 3.62 (t, *J* = 4.4 Hz, 2H, *H*-2′), 3.50–3.53 (m, 2H, *H*-3′), 2.38 (s, 3H, –CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 160.7, 156.4, 141.55, 141.47, 132.4, 129.7, 116.9, 99.1, 78.6, 73.9, 72.0, 61.6, 22.0; FAB-MS: *m/z* 301 $[M+H]^+$, 323 $[M+Na]^+$; HRMS: Calcd. for $C_{15}H_{17}N_4O_3^+$ 301.1295. Found 301.1304; IR: 2229.15 cm^{-1} (–C≡C–).

5-[(4-Pentylphenyl)ethynyl]-1-[(2-hydroxyethoxy)methyl] -1,2,4-triazole-3-carboxamide (**1d**)*:* White solid (92%). ^{1}H NMR (300 MHz, DMSO-d_6 + D_2O): δ 7.58 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.30 (d, *J* = 8.0 Hz, 2H, Ar*H*), 5.68 (s, 2H, *H*-1′), 3.58 (t, *J* = 5.0 Hz, 2H, *H*-2′), 3.47 (t, *J* = 5.0 Hz, 2H, *H*-3′), 2.58 (t, *J* = 7.7 Hz, 2H, –CH_2), 1.51–1.55 (m, 2H, –CH_2–), 1.22–1.26 (m, 4H, –C *H*$_2$–), 0.80 (t, *J* = 6.6 Hz, 3H, –CH_3); ^{13}C NMR (75 MHz, DMSO-d_6):δ 160.5, 157.5, 146.5, 140.8, 132.7, 129.7, 117.3, 98.0, 78.7, 75.2, 72.1, 60.5, 35.8, 31.5, 30.9, 22.6, 14.6; FAB-MS: *m/z* 357 $[M+H]^+$, 379 $[M+Na]^+$; HRMS: Calcd for $C_{19}H_{25}N_4O_3^+$ 357.1921. Found 357.1929; IR: 2230.07 cm^{-1} (–C≡C–).

5-[(4-Trifluoromethyl-phenyl)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1e**)*:* White solid (75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.08 (br s, 1H, –C(O)N *H*), 7.98 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.90 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.78 (br s, 1H, –C(O)N*H*), 5.78 (s, 2H, *H*-1′), 4.76 (t, *J* = 5.3 Hz, –O *H*), 3.63 (t, *J* = 5.1 Hz, 2H, *H*-2′), 3.49–3.54 (m, 2H, *H*-3′); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.5, 157.3, 140.3, 133.6, 131.2 (q, $^2J_{CF}$ = 32.0 Hz), 126.6, 124.3 (q, $^1J_{CF}$ = 270.4 Hz), 124.2, 96.0, 78.9, 77.3, 72.0, 60.3; FAB-MS: *m/z* 355 $[M+H]^+$, 377 $[M+Na]^+$; HRMS: Calcd for $C_{15}H_{14}F_3N_4O_3^+$ 355.1013. Found 355.1013; IR: 2233.78 cm^{-1} (–C≡C–).

5-[(4-Fluorophenyl)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1f**)*:* White solid (86%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.07 (br s, 1H, –C(O)N*H*), 7.80–7.85 (m, 2H, Ar*H*), 7.77 (br s, 1H, –C(O)N*H*), 7.39 (t, J_{HH} = 8.7 Hz, J_{FH} = 8.7 Hz, 2H, Ar*H*), 5.75 (s, 2H, *H*-1′), 4.78 (br s, 1H, –O *H*), 3.62 (t, *J* = 4.8 Hz, 2H, *H*-2′), 3.50–3.53 (m, 2H, *H*-3′); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.9 (d, $^1J_{CF}$ = 249.1 Hz), 160.4, 157.6, 140.5, 135.5 (d, $^3J_{CF}$ = 8.6 Hz), 117.2 (d, $^2J_{CF}$ = 23.4 Hz), 116.6, 96.6, 78.8, 75.5, 72.1, 60.5; FAB-MS: *m/z* 305$[M+H]^+$, 327 $[M+Na]^+$; HRMS: Calcd. for $C_{14}H_{14}FN_4O_3^+$ 305.1044. Found 305.1048; IR: 2230.09 cm^{-1} (–C≡C–).

5-[(3-Fluorophenyl)ethynyl]-1-[(2-hydroxyethoxy)methyl] -1,2,4-triazole-3-carboxamide (**1g**)*:* White solid (77%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.07 (br s, 1H, –C(O)N *H*), 7.77 (br s, 1H, –C(O)N *H*), 7.67 (d, *J* = 8.7 Hz, 1H, Ar*H*), 7.54–7.60 (m, 2H, Ar*H*), 7.43–7.49 (m, 1H, Ar*H*), 5.77 (s, 2H, *H*-1′), 4.76 (t, *J* = 5.4 Hz, –O*H*), 3.63 (t, *J* = 4.8 Hz, 2H, *H*-2′), 3.49–3.54 (m, 2H, *H*-3′); ^{13}C NMR (150 MHz, DMSO-d_6): δ 162.5 (d, $^1J_{CF}$ = 243.6 Hz), 160.3, 157.6, 140.3, 132.0 (d, $^3J_{CF}$ = 8.7 Hz), 129.2, 122.0 (d, $^3J_{CF}$ = 9.9 Hz), 119.4 (d, $^2J_{CF}$ = 24.2 Hz), 118.9 (d, $^2J_{CF}$ = 19.7 Hz), 96.0, 78.8, 76.3, 72.1, 60.5; HRMS: Calcd for $C_{14}H_{14}FN_4O_3^+$ 305.1044. Found 305.1043; IR: 2233.78 cm^{-1} (–C≡C–).

5-[(2-Fluorophenyl)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1h**)*:* White solid (85%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.10 (br s,1H, –C(O)N*H*), 7.80–7.85 (m, 1H, Ar*H*), 7.77 (br s, 1H, –C(O)N *H*), 7.64–7.66 (m, 1H, Ar*H*), 7.35–7.48 (m, 2H, Ar*H*), 5.74 (s, 2H, *H*-1′), 4.76 (t, *J* = 5.6 Hz, –O*H*), 3.63 (t, *J* = 5.1 Hz, 2H, *H*-2′), 3.49–3.54 (m, 2H, *H*-3′); ^{13}C NMR (150 MHz, DMSO-d_6): δ 163.0 (d, $^1J_{CF}$ = 251.3 Hz), 160.3, 157.7, 140.2, 134.7, 134.0, 125.9, 116.8 (d, $^2J_{CF}$ = 19.8 Hz), 108.7 (d, $^2J_{CF}$ = 15.3 Hz), 91.0, 80.3, 78.9, 72.2, 60.4; FAB-MS: *m/z* 305 $[M+H]^+$, 327 $[M+Na]^+$; HRMS: Calcd for $C_{14}H_{14}FN_4O_3^+$ 305.1044. Found 305.1046; IR: 2230.12 cm^{-1} (–C≡C–).

5-[(1-Cyclopentanol)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1i**)*:* White solid (97%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.00 (br s, 1H, –C(O)N *H*), 7.72 (br s, 1H, –C(O)N *H*), 5.74 (s, 1H, –O *H*), 5.62 (s, 2H, *H*-1′), 4.76 (t, *J* = 5.6 Hz, 1H, –O *H*), 3.58 (t, *J* = 4.4 Hz, 2H, *H*-2′), 3.48–3.52 (m, 2H, *H*-3′), 1.95 (br s, 4H, –C H_2–), 1.65–1.86 (m, 4H, –C H_2–); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.4, 157.4, 140.7, 104.2, 78.4, 73.4, 72.1, 68.6, 60.4, 42.1, 23.7; FAB-MS: *m/z* 295 $[M+H]^+$, 317 $[M+Na]^+$; HRMS: Calcd for $C_{13}H_{19}N_4O_4^+$ 295.1401. Found 295.1402; IR: 2237.00 cm^{-1} (–C≡C–).

5-[(1-Cyclohexanol)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1j**)*:* White solid (91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.01 (br s, 1H, –C(O)N *H*), 7.71 (br s, 1H, –C(O)N*H*), 5.87 (s, 1H, –O*H*), 5.62 (s, 2H, *H*-1′), 4.75 (t, *J* = 5.1 Hz, 1H, –O*H*), 3.58 (t, *J* = 4.8 Hz, 2H, *H*-2′), 3.47–3.52 (m, 2H, *H*-3′), 1.27–1.91 (m, 10H, –C H_2–); ^{13}C NMR (150 MHz, DMSO-d_6): δ 159.3, 156.2, 139.5, 103.0, 77.4, 70.9, 68.5, 66.7, 59.3, 38.4, 24.2, 22.0; ESI-MS: *m/z* 331.1 $[M+Na]^+$; HRMS: Calcd for $C_{14}H_{21}$ $N_4O_4^+$ 309.1557. Found 309.1560; IR: 2243.43 cm^{-1} (–C≡C–).

5-[(5-Chloropentyl)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (**1k**)*:* White solid (93%). ^{1}H NMR (300 MHz, DMSO-d_6): δ7.98 (br s, 1H, –C(O)N*H*), 7.70 (br s, 1H, –C(O)N *H*), 5.61 (s, 2H, *H*-1′), 4.73 (t, *J* = 5.1 Hz, 1H, –O*H*), 3.78 (t, *J* = 6.2 Hz, 2H, –CH_2–), 3.56 (t, *J* = 4.4 Hz, 2H, *H*-2′), 3.46–3.51 (m, 2H, *H*-3′), 2.74 (t, *J* = 6.9 Hz, 2H, –C H_2–), 2.01–2.10 (m, 2H, –CH_2–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.8, 156.2, 141.1, 99.4, 78.5, 71.9, 67.6, 61.6, 43.5, 30.5, 17.1; FAB-MS: *m/z* 287 $[M+H]^+$, 309 $[M+Na]^+$; HRMS: Calcd for $C_{11}H_{15}ClN_4$ NaO_3^+ 309.0725. Found 309.0727; IR: 2248.32 cm^{-1} (–C≡C–).

5-[(1-Cyclohexene)ethynyl]-1-[(2-hydroxyethoxy)methyl]-1,2,4-triazole-3-carboxamide (1l)*:* Colorless oil (90%). ^{1}H

NMR (300 MHz, DMSO-d_6): δ 8.02 (br s, 1H, –C(O)N *H*), 7.71 (br s, 1H, –C(O)N *H*), 6.52 (s, 1H, –C *H*═C–), 5.62 (s, 2H, *H*-1′), 4.75 (t, J = 5.1 Hz, 1H, –O *H*), 3.57 (t, J = 4.7 Hz, 2H, *H*-2′), 3.48–3.52 (m, 2H, *H*-3′), 2.17–2.22 (m, 4H, –C H_2–), 1.58–1.65 (m, 4H, –C H_2–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.0, 156.3, 141.7, 141.4, 118.8, 100.7, 78.4, 72.0, 71.9, 61.5, 28.4, 26.1, 22.1, 21.3; ESI-MS: *m/z* 291.0 $[M+H]^+$; HRMS: Calcd for $C_{14}H_{19}N_4O_3^+$ 291.1452. Found 291.1457; IR: 2214.48 cm^{-1} (–C≡C–).

14. Negishi, E. I.; Anastasia, L. *Chem. Rev.* **2003**, *103*, 1979.
15. Anastasia, L.; Negishi, E. I. *Org. Lett.* **2001**, *3*, 3111.
16. Single crystals of product **1a** suitable for X-ray crystallographic analysis were obtained via slow evaporation of CH_2Cl_2–CH_3OH solution. Crystallographic data of **1a**: colorless, monoclinic space group P 21/c, Z = 8, a = 17.9247 (9), b = 9.1151 (5), c = 18.0680 (9) Å, α = 90.00, β = 106.6130 (10), γ = 90.00, V = 2828.8 (3) Å^3, R ($F^2 > 2\sigma F^2$) = 0.0553 and wR = 0.1269 ($w = 1/[\sigma^2(F_o^2) + (0.0637P)^2 + 0.4295P]$, $P = (F_o^2 + 2F_c^2)/3$). The detailed X-ray structure data have been deposited in the Cambridge Crystallographic Data Center with deposition No. CCDC 680416. Copy of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ UK (Email: deposit@ccdc.cam.ac.uk).
17. Single crystals of product **1f** suitable for X-ray crystallographic analysis were obtained via slow evaporation of CH_2Cl_2–CH_3OH solution. Crystallographic data of **1f**: colorless, triclinic space group $P-1$, Z = 2, a = 5.1149 (6), b = 8.4420 (9), c = 17.0589 (18) Å, α= 93.615 (2), β = 90.062 (2), γ = 101.108 (2), V = 721.30 (14) Å^3, R ($F^2 > 2\sigma F^2$) = 0.0531 and wR = 0.1479 ($w = 1/[\sigma^2(F_o^2) + (0.1018P)^2 + 0.0000P]$, $P = (F_o^2 + 2F_c^2)/3$). The detailed X-ray structure data have been deposited in the Cambridge Crystallographic Data Center with deposition No. CCDC 680417. Copy of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ UK (Email: deposit@ccdc.cam.ac.uk).
18. (a) Paeshuyse, J.; Kaul, A.; De Clercq, E.; Rosenwirth, B.; Dumont, J. M.; Scalfaro, P.; Bartenschlager, R.; Neyts, J. *Hepatology* **2006**, *43*, 761; (b) Coelmont, L.; Paeshuyse, J.; Windisch, M. P.; De Clercq, E.; Bartenschlager, R.; Neyts, J. *Antimicrob. Agents Chemother.* **2006**, *50*, 3444.
19. *Anti-HCV Assay in Huh-5-2 cells.* Huh-5-2 cells were seeded at a density of 5000 per well in a tissue culture-treated white 96-well view plate (Packard, Canberra, Canada) in complete Dulbecco's modified Eagle's medium (DMEM) supplemented with 250 μg/mL G418. After incubation for 24 h at 37 °C (5% CO_2) medium was removed and threefold serial dilutions in complete DMEM (without G418) of the test compounds were added in a total volume of 100 μL. After 4 days of incubation at 37 °C, cell culture medium was removed and luciferase activity was determined using the Steady-Glo luciferase assay system (Promega, Leiden, The Netherlands); the luciferase signal was measured using a Luminoskan ascent (Thermo, Vantaa, Finland).

Anti-HCV Assay in Huh-9-13 cells. Huh-9-13 cells were seeded at a density of 5000 cells per well in 96-well cell culture plates in complete DMEM supplemented with 1,000 μg/mL G418. After 24 h incubation at 37 °C, cell culture medium was removed and threefold serial dilutions of the test compounds in complete DMEM without G418 were added in a total volume of 100 μL. After 4 days incubation at 37 °C, cell culture fluid was removed and monolayers were washed once with phosphate-buffered saline. Cells were lysed in 350 μL RLT buffer (Qiagen, Venlo, The Netherlands) according to the manufacturer's instruction. Lysates were used to determine the amount of HCV replicon RNA by means of quantitative real-time PCR. The values of EC_{50} were calculated as the concentration of compound that caused a 50% reduction in HCV RNA levels compared to that of the untreated control.
Anti-HCV Assay in Huh-6 cells. Huh-6 cells were seeded at a density of 15,000 cells per well in 96-well cell culture plates as described for Huh 9-13. After a 3-day incubation period at 37 °C, cells were lysed in cells-to-cDNA lysis buffer (Ambion, Cambridgeshire, United Kingdom), and lysates were used to determine the amount of HCV replicon RNA by means of quantitative real-time PCR. The values of EC_{50} were calculated as the concentration of compound that caused a 50% reduction in HCV RNA levels compared to that of the untreated control.
20. Cytotoxicity of **1a** and **1f** was further evaluated on the MiaPaCa, Capan-2 and PC-3 cells using MTT assay. No significant toxicity was observed at concentrations even up to 200 μM (Xia, Y. et al., unpublished results).
21. *Synthesis of* **5***:* **1f** (31.8 mg, 0.105 mmol) and 10% Pd/C (11 mg) were suspended in 5 mL of CH_3OH and the obtained mixture was stirred under atmospheric hydrogen at room temperature for 24 h. The reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was dried in vacuo to afford product.
Compound **5**: White solid (30.8 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ *7.09–7.14 (m, 2H, ArH)*, 6.94–7.00 (m, 3H, Ar*H* and –C(O)N*H*), 5.75 (br s, 1H, –C(O)N*H*), 5.37 (s, 2H, *H*-1′), 3.66–3.69 (m, 2H, *H*-2′), 3.59–3.62 (m, 2H, *H*-3′), 3.12 (s, 4H, –C H_2–), 1.80 (t, J = 5.6 Hz, –O*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.9 (d, $^1J_{CF}$ = 243.8 Hz), 161.3, 157.7, 155.3, 135.7, 130.1(d, $^3J_{CF}$ = 7.4 Hz), 115.7 (d, $^2J_{CF}$ = 21.3 Hz), 78.0, 71.3, 61.6, 33.0, 28.3; HRMS: Calcd for $C_{14}H_{18}FN_4O_3^+$ 309.1357. Found 309.1353.