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Bitriazolyl acyclonucleosides synthesized via Huisgen reaction using internal alkynes show antiviral activity against tobacco mosaic virus

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ABSTRACT

A family of novel bitriazolyl acyclonucleosides were synthesized using a simple and convenient one-step synthetic procedure via the Huisgen reaction by addition of NaN_3 onto triazole nucleosides bearing internal alkynyl groups introduced at the 5-position of the triazole ring. Some of the compounds exhibited interesting antiviral activity against tobacco mosaic virus, demonstrating the importance of the bitriazolyl motif for the observed antiviral activity.

In our ongoing program to develop novel triazole nucleoside analogs with biologically interesting activity,[1–10] we have recently discovered a novel family of bitriazolyl compounds,[5,6,9] in particular bitriazolyl nucleosides **A** and **B** (Scheme 1),[5,6] exhibiting potent antiviral activity against tobacco mosaic virus (TMV)—the most prevalent pathogen affecting tobacco plants and causing massive crop reduction. The identified hit compounds bear a bitriazolyl motif in which the two triazole rings are quasi co-planar via C–N bond connection, yielding an expanded and enlarged conjugated aromatic system.[5,6,9] This structural feature may favor the binding of bitriazolyl nucleoside analogs to their biological targets via stronger interactions offered by the larger aromatic binding surface together with broad H-bonding capacity.[10]

The bitriazolyl nucleoside **A** compounds were conveniently synthesized in very good yields via Huisgen reaction using 3-azidotriazole nucleosides **1** and various alkynes (**I** in Scheme 1).[5,6] However, the synthesis of their structural isomers **B** via Huisgen reaction using the corresponding 5-azidotriazole nucleosides **2** was problematic (**II** in Scheme 1): either no bitriazolyl products were identified as was the case for the ribonucleosides[6] or very low product yields were obtained as was found for the acyclonucleosides.[5] This can be mainly attributed to the unfavorable electronic properties and steric hindrance of the corresponding 5-azidotriazole nucleosides **2**, which prevent them from undergoing Huisgen reaction under the Cu-catalyzed experimental conditions.[5,6,11] In addition, the C–N bond linking the 1,2,3-triazolyl ring to the 1,2,4-triazole cycle in **B** seems to be weakened, making the 1,2,3-triazoyl moiety readily displaceable with nucleophilic agents.[5,12] This can be also a direct consequence of both the electronic properties and steric congestion presented in **B**.

To obtain stable analogs of bitriazolyl nucleosides **B**, we were interested in developing bitriazolyl nucleosides **C** (Scheme 1) in which the 1,2,3-triazolyl ring is connected to the 1,2,4-triazole ring via a C–C bond instead of the C–N bond thus conferring better stability. The synthesis of this new family of bitriazolyl nucleosides **C** can be also envisaged via Huisgen reaction by the addition of NaN_3 onto the triazole nucleosides **3** bearing internal alkynyl groups introduced at the 5-position of the triazole ring (**III** in Scheme 1). Indeed, this strategy offers a simple and convenient one-step synthetic procedure. Here, we report the synthesis of this new family of bitriazolyl nucleosides **C**, specifically bitriazolyl acyclonucleosides **4** (Tables 1 and 2) and their biological activity in terms of antiviral activity against tobacco mosaic virus.

We first carried out a Huisgen reaction starting with the 5-alkynyltriazole acyclonucleoside **3a** and NaN_3 in the mixed solvent

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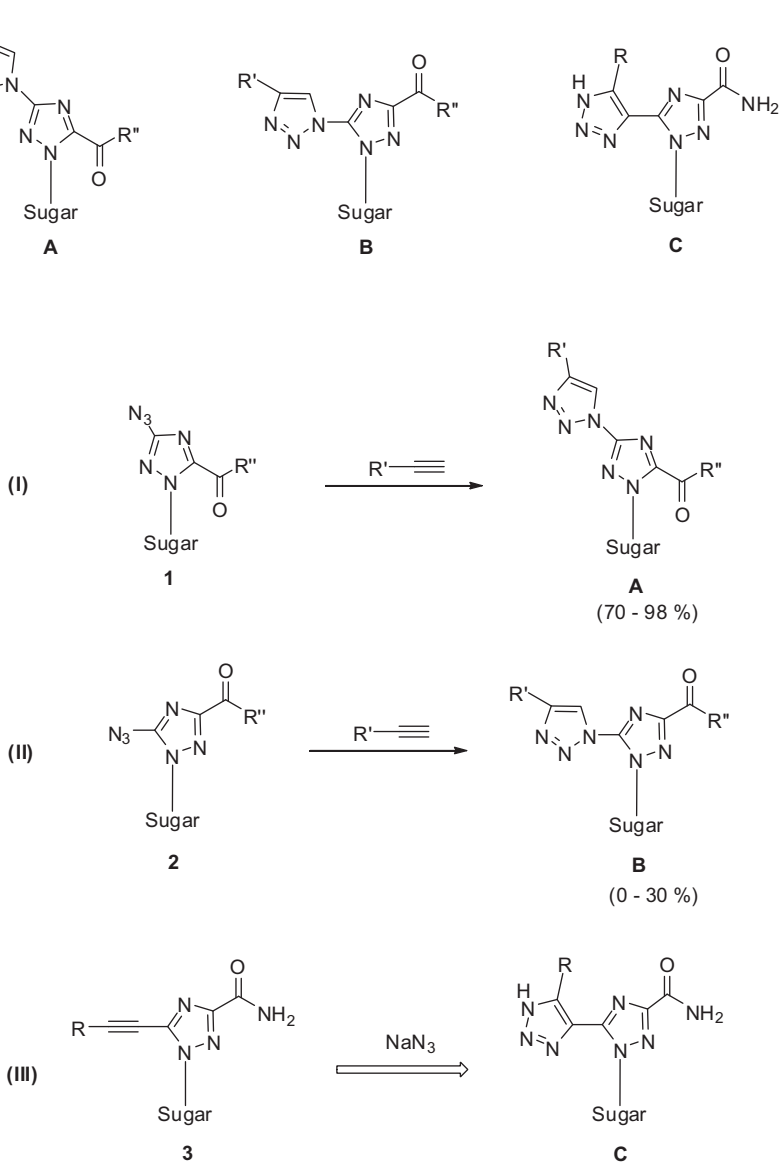
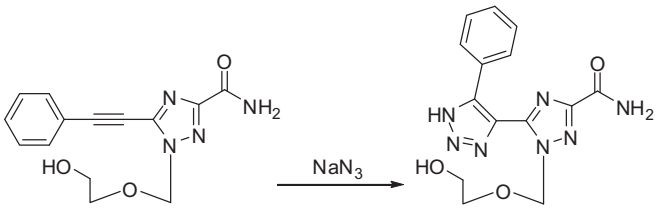


Scheme 1. Bitriazolyl nucleosides **A**, **B**, and **C**, and their synthesis via Huisgen reaction starting with the corresponding triazole nucleosides **1** (**I**), **2** (**II**), and **3** (**III**), respectively.

system THF/H_2O (1/3) at 40 °C. Unfortunately, no corresponding product was identified and we observed an almost complete recovery of the starting material (Table 1, entry 1). By raising the temperature to 90 °C, while the reaction could deliver the desired bitriazolyl nucleoside **4a** in satisfactory yields (Table 1, entry 2), the reaction time was relatively long (48 h) compared to conventional Huisgen reaction. Microwave irradiation was able to considerably reduce this reaction time to 1 h but offered no beneficial effect on the reaction yield (Table 1, entries 2 and 3). This is in line with our previous findings that no substantial improvement was observed with microwave irradiation for the synthesis of bitriazolyl compounds via Huisgen reaction,[5,6] probably due to the presence of the triazole unit adjacent to the triple bond, as microwave irradiation has been frequently reported to improve the Huisgen reaction significantly.[11,13] Further examination of alternative solvents (Table 1, entries 4–9) led us to identify DMF as the solvent of choice for this reaction: the desired product **4a** could be obtained with satisfactory yield within a short reaction time (1.5 h) under simple oil bath heating in DMF (Table 1, entry 9); whereas in other solvent systems, the reaction did not go to completion even after a relatively long reaction time and large amounts of starting material were recovered (Table 1, entries 4–8). It should be mentioned that polar solvents such as DMF and DMSO are often employed in Huisgen reaction when NaN_3 is used as the azido reagent.[14,15] This may be ascribed to the fact that polar solvents can better solubilize NaN_3 and, at the same time, may induce the internal alkynes to undergo polarization, thus favoring the corresponding [3+2] Huisgen cycloaddition. Moreover, the bitriazolyl compounds are highly polar products thus requiring polar solvents such as DMF to stabilize both the reaction intermediates and products. Attempts to use either an excess of NaN_3 (Table 1, entry 10) or microwave irradiation (Table 1, entry 11) did not yield significant benefit to the reaction time but rather decreased the reaction yields in DMF. Consequently, to synthesize **4**, we carried out the Huisgen reaction under the above optimized conditions, namely using 1.2 equiv NaN_3 in DMF with oil bath heating at 90 °C (Table 2).

The starting materials for the Huisgen reaction, 5-alkynylyltriazole nucleosides **3**, were prepared using the protocol previously developed in our laboratories.[4] The corresponding bitriazolyl acy-

Table 1
Optimization of the Huisgen reaction to synthesize bitriazolyl acyclonucleoside **4a** using 5-phenylethynyltriazole acyclonucleoside **3a**[a]



3a **4a**

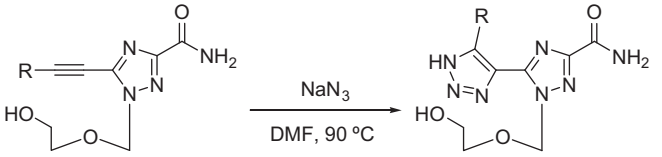
Entry	NaN_3	Solvent	Heating	Temperature	Time (h)	Yield (%) **4a** (**3a**)
1	1.2 equiv	THF/H_2O (1/3)	Oil bath	40 °C	48	0 (80)
2	1.2 equiv	THF/H_2O (1/3)	Oil bath	90 °C	48	50 (10)
3	1.2 equiv	THF/H_2O (1/3)	Microwave	90 °C	1	48 (18)
4	1.2 equiv	THF/H_2O (3/1)	Oil bath	90 °C	48	33 (63)
5	1.2 equiv	Dioxane/H_2O (1/3)	Oil bath	90 °C	48	22(62)
6	1.2 equiv	MeOH/H_2O (1/3)	Oil bath	90 °C	48	22 (44)
7	1.2 equiv	MeOH	Oil bath	90 °C	48	26 (45)
8	1.2 equiv	CH_3CN	Oil bath	Reflux	24	0 (33)[b]
9	1.2 equiv	DMF	Oil bath	90 °C	1.5	66[c]
10	5.0 equiv	DMF	Oil bath	90 °C	1.5	54[c]
11	1.2 equiv	DMF	Microwave	90 °C	0.5	38[c]

[a] Position of the triazole NH is arbitrarily assigned since we cannot reliably determine the tautomeric structure.
[b] Only 33% starting material was recovered. Many side products were observed on TLC plate and could not be isolated.
[c] No starting material was recovered. Side products were observed on TLC plate and could not be isolated.

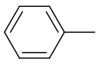
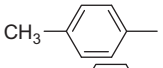
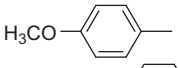
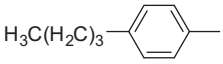
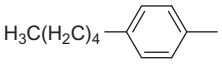
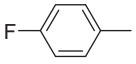
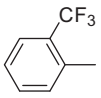
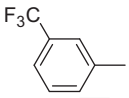
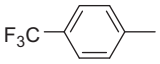
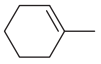
clonucleosides **4** were obtained mostly in good and satisfactory yields using the above mentioned conditions (Table 2).[16] This simple one-step and easy-to-perform synthetic procedure is clearly advantageous over the previously reported two-step synthetic procedure for **B**.[5] The reaction yields were not significantly affected by the presence of the electron-donating (Table 2, entry 3) or electron-withdrawing (Table 2, entries 6–9) groups on the phenyl ring adjacent to the triple bond, although reaction completion was achieved more rapidly with the electron-withdrawing groups (Table 2, entries 6–9). The reaction furnished similar yields when either heterocyclic arylacetylene (Table 2, entry 10) or alkylacetylene (Table 2, entry 11) were employed. Furthermore, no considerable steric effect was observed (Table 2, entries 7–9). Finally, the products obtained showed an increased stability compared to that previously observed with **B**, since the 1,2,3-triazolyl ring could not be readily displaced from the 1,2,4-tiazolyl ring in **4**.

The synthesized bitriazolyl acyclonucleosides **4** were tested for their antiviral activity against TMV using the conventional half-leaf juice rubbing method[17] with ribavirin as the positive control and water as the negative control. Among the tested compounds, **4a**, **4c**, **4e**, and **4k** showed levels of anti-TMV activity either similar to or better than ribavirin, the standard reference for anti-TMV assay (Table 3). In addition, these active compounds shared some similar structural features to the previously identified active bitriazolyl nucleosides.[5,6,9] Collectively, these findings demonstrate the importance of bitriazolyl nucleoside derivatives as structural motifs in the search for candidates with potent antiviral activity against TMV.

Table 2
Synthesis of bitriazolyl acyclonucleosides **4** via Huisgen reaction using NaN_3 and various 5-alkynyltriazole acyclonucleosides **3**[a]



3 **4**

Entry	R	Product	Yield (%)	Reaction time (h)
1		**4a**	66	1.5
2		**4b**	50	5
3		**4c**	59	5
4		**4d**	50	4
5		**4e**	50	4
6		**4f**	63	1
7		**4g**	52	0.5
8		**4h**	55	0.5
9		**4i**	53	0.5
10		**4j**	47	5
11	$H_3C(H_2C)_4$–	**4k**	50	4
12		**4l**	40[b]	6

[a] Position of the triazole NH is arbitrarily assigned since we cannot reliably determine the tautomeric structure.
[b] Reaction was carried out at 120 °C, and many by-products were observed by TLC.

Table 3
Antiviral activity of bitriazolyl acyclonucleosides **4** against tobacco mosaic virus using ribavirin as control reference

Compound	Anti-TMV activity (%)
4a	36 ± 3
4b	28 ± 9
4c	39 ± 4
4d	22 ± 3
4e	44 ± 4
4f	29 ± 1
4g	17 ± 1
4h	16 ± 2
4i	30 ± 1
4j	14 ± 5
4k	34 ± 7
4l	23 ± 1
Ribavirin	39 ± 8

We further carried out toxicity prediction on the acute and mutagenic toxicity for the identified active compounds **4a**, **4c**, **4e**, and **4k** using the programs of CISOC-PSAT[18]and CISOC-PSMT,[19] respectively. Acute toxicity describes the adverse effects of a

Table 4
Predicted results of the acute toxicity, mutagenic toxicity, and log *P* values for the active compounds **4a**, **4c**, **4e**, and **4k** using the CISOC-PSAT, CISOC-PSMT, and CISOC-log *P* programs, respectively

Compound	Acute toxicity (CISOC-PSAT)	Mutagenic toxicity (CISOC-PSMT)			log *P* (CISOC-log *P*)
		Predictability	Mutagenicity possibility	Mutagenicity impossibility	
4a	4.05	96%	0.01	0.21	0.62
4c	4.12	96%	0.01	0.28	0.64
4e	4.17	96%	0.01	0.49	2.86
4k	4.39	96%	0.01	0.95	0.38

substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 h),[20] whereas mutagenic toxicity reveals the extent to which a compound is susceptible to induce abnormal mutation. Both acute toxicity and mutagenic toxicity are two important indicators of the toxic potential of a chemical compound. It is therefore important to evaluate these two parameters for further lead optimization and development purposes. In toxicology, acute toxicity could be classified into five levels: severe toxic (rat, oral, LD_{50} <1 mg/kg, $1 \leqslant$ predicted value <2), high toxic (rat, oral, LD_{50}: 1–49 mg/kg, $2 \leqslant$ predicted value <3), medium toxic (rat, oral, LD_{50}: 50–499 mg/kg, $3 \leqslant$ predicted value <4), low toxic (rat, oral, LD_{50}: 500–4999 mg/kg, $4 \leqslant$ predicted value <5), and tiny toxic (rat, oral, LD_{50}: >5000 mg/kg, predicted value $\geqslant 5$).[21] For mutagenic toxicity, if the predictability is >80% and the mutagenicity possibility is less than the mutagenicity impossibility, the compound is considered not mutagenic. Gratifyingly, the results of our calculations reveal that all the four active compounds have no notable acute toxicity and are devoid of mutagenic toxicity (Table 4).

We next evaluated the Log *P* values using the program of CISOC-log *P*.[22] log *P* is an index of molecular hydrophobicity, a parameter which affects the compound bioavailability, the interaction with the biological targets, and the metabolism, as well as the toxicity. It has become one of the key parameters used to study the fate and behavior of bioactive compounds. According to Lipinski's rule of five, log *P* should optimally be <5.[23] The prediction results showed that log *P* values of the four identified active compounds are between 0.38 and 2.86 (Table 4), confirming that they represent promising candidates for further lead optimization and development.

In conclusion, a new series of bitriazolyl acyclonucleosides **C**, the structural isomers of our previously reported bitriazolyl acyclonucleosides **A** and **B**, have been synthesized via a one-step Huisgen cycloaddition using NaN_3 and various internal alkynes of 5-alkynylyltriazole acyclonucleosides. The reaction is straightforward via an easy-to-perform procedure and gives the corresponding products in good yields. Similar to the previously identified active hits,[5,6,9] some of the newly synthesized bitriazolyl compounds showed interesting anti-TMV activity and were devoid of any notable toxicity, confirming the importance of the bitriazolyl motif in the observed antiviral activity against TMV. We are now actively working towards defining the detailed structure/activity relationships of this family of compounds and their related biological activity against tobacco mosaic virus and other pathogenic viruses.

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Supplementary data

Supplementary data (experimental details, ^1H NMR and ^{13}C NMR spectra of all the new compounds described) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.141.

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