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Journal of Atlantic Research and Technology

Synthesis and NMR Study of Novel Acyclonucleosides Derived from Pyrazolo[3,4-d][1,2,3]triazines with Alkyl Chains of Acyclovir and HBG

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ARTICLE INFO

Article history:
Received 14th January, 2024
Received in revised form 18th March, 2024
Accepted 24th March, 2024
Available online 1sh April, 2024

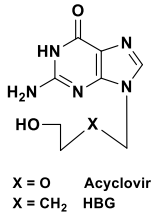
Keywords:
Heterocyclic compounds
Acyclonucleosides
NMR
pyrazolo[3,4-d][1,2,3]triazine

ABSTRACT

The present work describes the synthesis, the NMR study of new acyclonucleosides of pyrazolo[3,4-d][1,2,3]triazines, with the alkyl chains of acyclovir and HBG known for their potent anti-herpetic activity. To obtain these acyclonucleosides. Firstly, we synthesized 4-Hydroxy-7H-pyrazolo[3,4-d][1,2,3]triazines 7 from 3-aminocarboxamide 6 as precursors. Subsequently, we conducted the alkylation reaction of 4-hydroxy-7H-pyrazolo[3,4-d][1,2,3]triazine 7 separately with (2-acetoxyethoxy)methyl bromide 8 and 4-bromobutyl acetate 9 under the conditions of solid-liquid phase transfer catalysis. All structures of the synthetic products were identified by NMR 1D (1H NMR and 13C NMR), 2D NMR (HMQC and HMBC), and mass spectra.

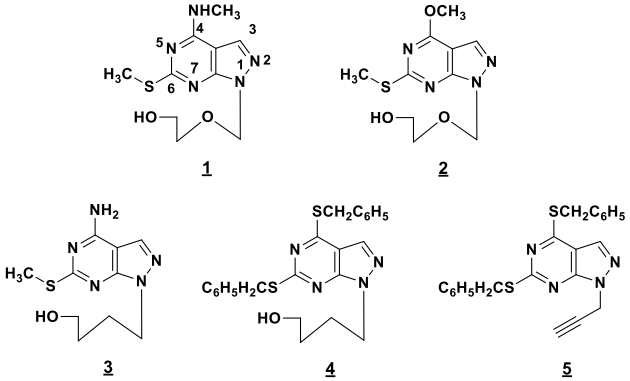
1. Introduction

Since the discovery of 9-[(2-hydroxyethoxy)methyl]guanine [1] (acyclovir or ACV) and its clinical use in the treatment of herpetic diseases, a large number of acyclonuleosides with modifications on the pseudosugar and/or heterocyclic moiety have been synthesized and tested against different types of virus. 9-(4-hydroxybutyl)guanine (HBG) [2,3] has shown significant antiherpetic activity (Figure 1).



- Figure 1 -

In literature, several acyclonucleoside pyrazolo[3,4-d]pyrimidine analogs of acyclovir and HBG were prepared and tested by Taha and al., [4-7] against several virus types. Acyclonucleosides substituted at positions 4 and/or 6, such as compounds 1-5 (Figure 2), were among those shown to have interesting antiviral activity.



- Figure 2 -

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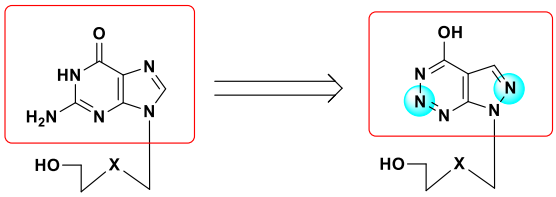
Based on this research, and aiming to develop novel acyclonucleosides with promising biological activities, we opted to modify the heterocyclic guanine base. We maintained acyclovir and HBG as the acyclic chains while incorporating 4-hydroxypyrazolo[3,4-d][1,2,3]triazine as the heterocyclic base (Figure 3).



- Figure 3 -

In the initial stage, the novel base 4-hydroxypyrazolo[3,4-d][1,2,3]triazine was synthesized through a four-step process starting from readily available and cost-effective compounds. Subsequently, products **10-13** were synthesized via alkylation reactions employing transfer catalysis (PTC). The characterization of these new products, **7** and **10-13** was based on their NMR ^{1}H, ^{13}C and mass spectra.

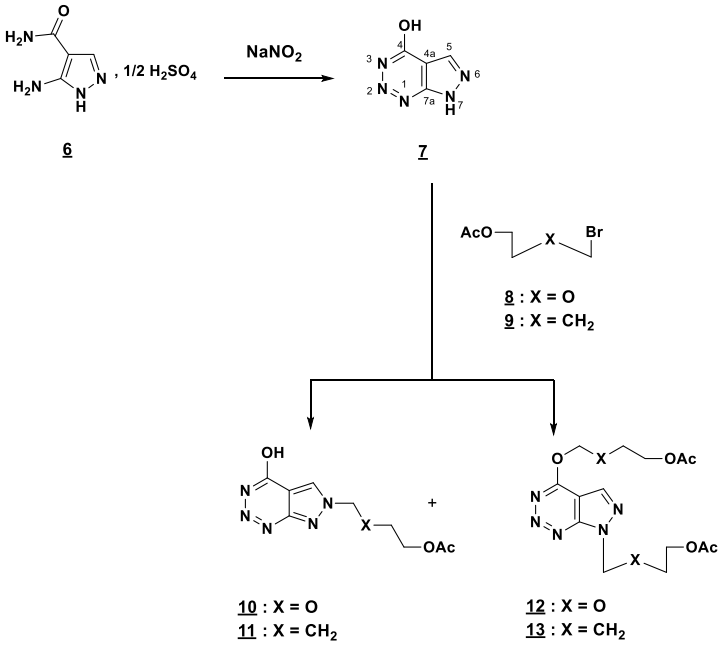
2. Material and methods

2.1. Measurements of spectral data

Melting points were taken in capillaries without correction, on an "Electrothermal Digital" apparatus, at the Agadir Faculty of Science. Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 F254 silica plates with fluorescent indicator. The spots were revealed by UV lamp (250 nm). Purifications and separations by chromatography on silica gel columns were carried out using Merck silica 0.063-0.2 nm (70-230 mesh ASTM). Proton nuclear magnetic resonance (1H-NMR) and 13C-NMR spectra were recorded at room temperature using a Bruker AC (250 MHz) instrument at the University of Montpellier 2, France and by a Bruker AC (400 MHz) instrument at Southern Research Institute, Birmingham, Alabama, USA. Chemical shifts (δ) are expressed in ppm relative to TMS taken as internal reference. Coupling constants (J) are given in Hertz, and the multiplicity of signals observed is indicated by a lower-case letter: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) and sl (broad singlet). Mass spectra (MS) were recorded at the Southern Research Institute, Birmingham, Alabama, USA and at the Institut de Chimie des Substances Naturelles in Paris, France on a JEOL JMX 300 DX instrument using the FAB ionization method in positive mode with either a glycerol-thioglycerol (GT) mixture (50/50, v/v) or 3-nitrobenzyl alcohol (NBA) as matrix.

2.2. Preparation of acyclonucleoside

Initially, we synthesized 4-Hydroxy-7H-pyrazolo[3,4-d][1,2,3]triazines from 3-amino-4-cyanopyrazole **6** which upon treatment with sodium nitrite resulted in 4-Hydroxypyrazolo[3,4-d][1,2,3]triazine **7** in good yield. Subsequently, we conducted the alkylation reaction of 4-hydroxy-7H-pyrazolo[3,4-d][1,2,3]triazine **7** separately with (2-acetoxyethoxy)methyl bromide **8** and 4-bromobutyl acetate **9** under the conditions of solid-liquid phase transfer catalysis (Scheme 1).

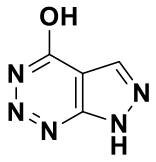


- Scheme 1 -

2.2.1. Synthesis of the compounds 7

Preparation of 4-hydroxy-7H-pyrazolo[3,4-d][1,2,3]triazine: 7

10 g (57.1 mmol) of compound 6 was dissolved in a 50 ml mixture of acetic acid and hydrochloric acid (50/50: v/v) under magnetic stirring at 0°C. After a few minutes, 4 g of sodium nitrite (NaNO2) solubilized in a minimum of cold distilled water was added portion by portion. After stirring for 2 hours, the precipitate formed was filtered and washed with distilled water to give the product 7 in the form of white crystals.



Yield: 98%. mp = decomposes at T > 100 °C. **^{1}H NMR** (Me2SO-d6) δ : 8,94 (s, 1H, $CH_{pyrazolic}$), 14.75 (s, 1H, NH). **^{13}C NMR** (Me_2SO-d_6) δ : 105.25(C4a), 130.38(C5), 154.60(C7a), 156.38(C4).

2.2.2. Synthesis of acyclonucleoside 4-hydroxypyrazolo[3,4-d]-[1,2,3] triazine 10-13.

General procedure

To a dry mixture of 0.89 mmol (160 mg) of the heterocyclic base, **7** and 425.29 mmol (100 mg) of

potassium carbonate in DMF (30 ml) added the 425.29 mmol (52 mg) of acyclonucleoside bromide **8** or **9** at 0°C. The mixture was stirred at room temperature for 16 hours. The reaction was monitored by TLC. After evaporation of the solvent under reduced pressure, the residue obtained was chromatographed on a silica gel column.

6-(4-acetoxyethoxymethyl)-4-hydroxy-6H-pyrazolo[3,4-d][1,2,3]triazine 10:

Yield: 23%. mp = 125-127 °C (ethanol). **^{1}H NMR** (Me_2SO-d_6) δ: 1.96 (s, 3H, CH_3CO), 3.79 and 4.12 (2m, 4H, OCH_2CH_2O), 5.78 (s, 2H, OCH_2N), 8.98 (s, 1H, $CH_{pyrazolic}$). **^{13}C NMR** (Me_2SO-d_6) δ : 20.47 (CH_3), 62.54 (CH_2), 67.54 (CH_2), 81.71 (CH_2), 106.27(C4a), 129.68 (C5), 154.29 (C7a), 156.68 (C4), 170.11(C=O). SM (FAB): m/z = 254.1 $[M + H]^+$.

6-(4-acetoxybutyl)-4-hydroxy-6H-pyrazolo[3,4-d][1,2,3]triazine 11:

Rdt : 30%. mp = 160-162 °C (ethanol). **^{1}H NMR** (Me_2SO-d_6) δ: 1.54 (m, 2H, CH_2CH_2N), 1.96 (m, 2H, CH_2CH_2N), 1.99 (s, 3H, CH_3CO), 4.01 (t, *J* = 6.4 Hz, 2H, $AcOCH_2CH_2$), 4.44 (t, *J* = 7.2 Hz, 2H, CH_2N), 8.82 (s, 1H, $CH_{pyrazolic}$). **^{13}C NMR** (Me_2SO-d_6) δ: 20.58 (CH_3), 24.94 (CH_2), 25.99 (CH_2), 52.88 (CH_2), 63.02 (CH_2), 105.69 (C4a), 128.76 (C5), 154.22(C7a), 156.46(C8), 170.26 (C=O). SM (FAB): m/z = 252.1 [M + H] +.

4,7-di(2-acetoxyethoxy)methyl-7H-pyrazolo[3,4-d][1,2,3]triazine 12:

Yield: 25%. **^{1}H RMN** (Me_2SO-d_6) δ: 1.94 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 3.78-3.85 (m, 4H, $(OCH_2CH_2OAc)_2$), 4.09-4.13 (m, 4H, $(OCH_2CH_2OAc)_2$), 5.79 (s, 2H, OCH_2O), 5.94 (s, 2H, NCH_2O), 8.53 (s, 1H, $CH_{pyrazolic}$). **^{13}C RMN** (Me_2SO-d_6) δ: 20.45 (CH_3), 20.55 (CH_3), 62.61 (CH_2), 62.85 (CH_2), 67.43 (CH_2), 67.43 (CH_2), 76.85 (CH_2), 77.87 (CH_2), 106.35(C4a), 135.43 (C5), 148.33 (C7a), 152.87 (C4), 170.15 (C=O), 170.18 (C=O). SM (FAB): m/z = 370.1 [M + H] +.

4,7-di(4-acetoxybutyl)-7H-pyrazolo[3,4-d][1,2,3]triazine 13:

Yield: 35%. **^{1}H RMN** (Me_2SO-d_6) δ: 1.56 (m, 4H, -CH_2CH_2OAc, - CH_2CH_2OAc), 1.72-1,87 (m, 4H, $OCH_2CH_2CH_2CH_2OAc$, $NCH_2CH_2CH_2CH_2OAc$), 1.95 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 2.11 (m, 4H, $OCH_2CH_2CH_2CH_2OAc$, $NCH_2CH_2CH_2CH_2OAc$), 4.04 (t, 2H, OCH_2), 4.46 (t, 2H, NCH_2), 8.82 (s, H, $CH_{pyrazolic}$). **^{13}C RMN** (Me_2SO-d_6) δ : 20.58 (CH_3), 20.59 (CH_3), 24.89 (CH_2), 24.94 (CH_2), 25.99 (CH_2), 28.24 (CH_2), 52.88 (CH_2), 63.02 (CH_2), 63.05 (CH_2), 64.78 (CH_2), 105.28(C4a), 128.74(C5), 154.29(C7a), 156.50 (C4), 170.26 (C=O), 170.80 (C=O). SM (FAB): m/z = 366.1 [M + H] + .

3. Results and discussion

The attempt to regioselectively prepare acyclonucleosides from 4-alkylsulfanyl-7H-pyrazolo[3,4-d][1,2,3]triazines posed several problems in terms of the sulfuration of the OH function in position 4 of 4-hydroxy-7H-pyrazolo[3,4-d][1,2,3]triazine, as the latter decomposes when heated[15,16].

To overcome this difficulty, we opted for the method of direct substitution in position 7 of the heterocyclic base **9** by the two acyclic chains of ACV and HBG, despite its several alkylation sites, which could lead to several regioisomers.

We therefore carried out the alkylation reaction of 4-hydroxy-7H-pyrazolo[3,4- d][1,2,3]triazine **7**, separately, with (2-acetoxyethoxy)methyl bromide **8** and 4-bromobutyl acetate bromide **9**, under the conditions of solid-liquid phase transfer catalysis (Scheme 1).

In all cases, the reaction leads to a mixture of two acyclonucleosides: monosubstituted (**10** and **11**) and disubstituted (**12** and **13**).

The structures of these acyclonucleosides **10-13** were identified on the basis of their ^{1}H NMR, ^{13}C NMR and mass spectral data.

The ^{1}H NMR spectrum of compound **10** reveals, in the acyclic part of the chain, a singlet at 1.96 ppm incorporating 3 protons, corresponding to the methyl protons of the acetyl group, two multiplets at approximately 3.79 ppm and 4.12 ppm attributed to the methylenic protons of the O-CH_2-CH_2-O group and a singlet at approximately 5.78 ppm, due to the O-CH_2-N methylenic protons.

Its ^{13}C NMR spectrum showed a signal at 20.47 ppm assigned to the methyl group, three signals at 62.54 ppm, 67.54 ppm and 81.17 ppm assigned to the methylene groups, and a signal at 170.11 ppm assigned to the carbonyl group.

The mass spectrum of product **10** showing a base peak at [M+H] + = 254.1 completes this analysis.

The ^{1}H NMR spectrum of the compound **11** reveals, in the acyclic chain, a singlet at 1.99 ppm incorporating 3 protons, corresponding to the methyl protons of the acetyl group, two multiplets at around 1.56 ppm and 1. 96 ppm attributed to the methylenic protons of the (N-CH_2-CH_2-CH_2-O) group, a triplet centered at 4.01 ppm and due to the methylenic protons (O-CH_2-) and a triplet centered at 4.44 ppm, corresponding to the methylenic protons (N-CH_2-).

Its ^{13}C NMR spectrum shows a signal at 20.58 ppm assigned to the methyl group, four signals between 24.94 ppm and 63.02 ppm assigned to the methylene groups, and a signal at 170.26 ppm relating to the carbonyl group.

The mass spectrum of product **11** shows a base peak at [M+H] + = 252.1.

In the case of product **12**, the ^{1}H NMR spectrum shows two singletons, one at 1.94 ppm and the other at 1.95 ppm, corresponding to the methyl protons of the two acetyl groups, two multiplets at around 3.78 ppm and 4. 13 ppm, incorporating 8 methylene protons from the two acyclic chains (ACO-CH_2-CH_2-O-CH_2-O) and (ACO-CH_2-CH_2-O-CH_2-N), two unshielded singletons at 5.79 ppm and 5.94 ppm attributed to the two types of methylene protons: (OCH_2-O) and (O-CH_2-N).

Its ^{13}C NMR spectrum is characterized by the presence of two signals at 20.45 ppm and 20 .55 ppm assigned to the two methyl groups, eight signals between 62.61 ppm and 76.85 ppm assigned to the methylene groups, two signals,

one at 170.15 ppm and the other at 170.18 ppm assigned to the carbonyl groups.

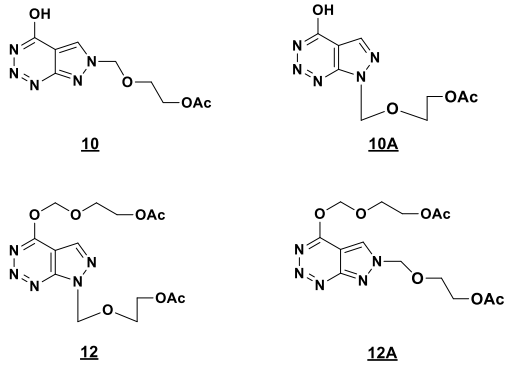
The mass spectrum of product **12** shows a base peak at [M+H] + = 370.1, indicating dialkylation.

The ^{1}H NMR spectrum of product **13** shows two singletons at around 2 ppm, corresponding to the methyl protons of the two acetyl groups, two multiplets at around 1.56 ppm and 1. 87 ppm, incorporating 8 methylene protons from the two acyclic chains ($ACOCH_2$-CH_2-CH_2-CH_2-O) and (ACO-CH_2-CH_2- CH_2-CH_2-N), two unshielded triplets at 4.02 ppm and 4.44 ppm, attributed to the two types of methylene protons: (O-CH_2-C) and (N-CH_2-C).

Its ^{13}C NMR spectrum is characterized by the presence of two signals at 20.58 ppm and 20 .59 ppm assigned to the two methyl groups, eight signals between 24.89 ppm and 64.78 ppm assigned to the methylene groups, and two signals, one at 170.26 ppm and other at 170.80 ppm, assigned to the carbonyl groups.

The mass spectrum of product **13** shows a base peak at [M+H]+ = 366.1, characteristic of a dialkylation reaction.

Analysis of the 1D ^{1}H NMR and ^{13}C NMR spectra did not allow the alkylation site of product **7** with the two acyclic chains of brominated alkylating agents **8** and **9** to be determined. The isolated products may therefore have structures **10** or **10A** for products monoalkylated in position 6 or 7 and **12** or **12A** for products dialkylated in position 4 and 6 or in position 4 and 7 (Figure 4).



- Figure 4 -

To determine the alkylation site with certainty, we used a 2D NMR study. Firstly, analysis of the heteronuclear correlation diagram (HMQC) of compound **10** (Spectrum 1), for example, enabled us to assign the 81.17 ppm signal to the C1' carbon of the methylene group and the 129.68 ppm signal to the pyrazole C5 carbon.

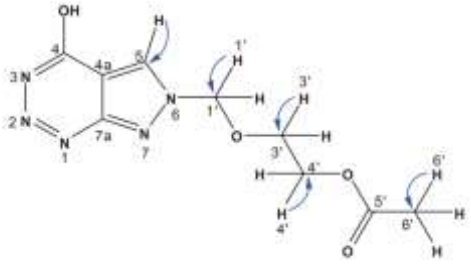
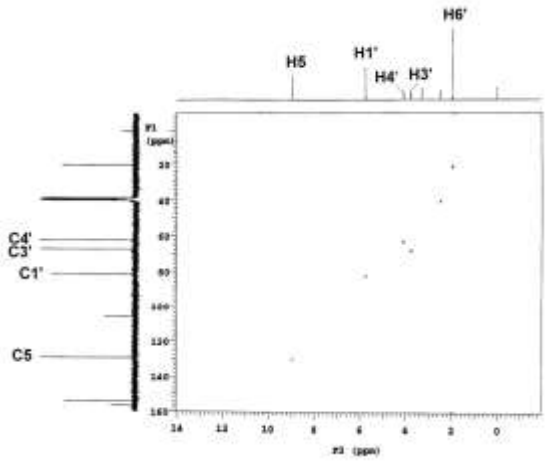


Figure 5: Illustration of the HMQC coupling of compound **10**



Spectrum 1: HMQC of product **10**

However, the HMQC technique is still insufficient to distinguish between these two structures and to determine the alkylation site 6 or 7. To do this, we analyzed the HMBC spectrum. The long-range couplings show coupling at 3 J between the protons attached to carbon C1' (δ: 5.78 ppm) and carbon C5, which appears at 129.68 ppm (Spectrum 2). This suggests that alkylation occurred on the nitrogen in position 6 and not on the nitrogen in position 7.

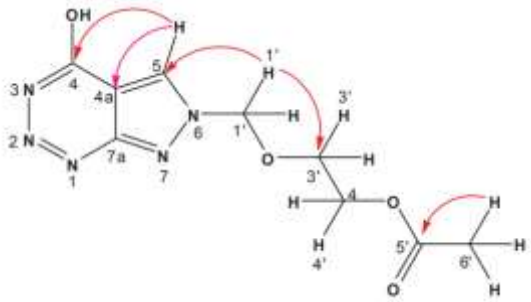
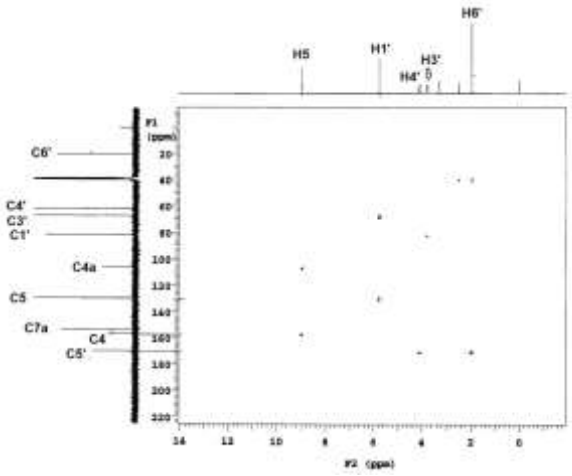


Figure 6: Illustration of the HMBC coupling of compound **10**



Spectrum 2 : HMBC of compound **10**

We proceeded, in the same way, to determine the two alkylation sites of dialkylated compounds **12** and **13** based on analysis of their HMQC and HMBC spectra. In the case

of compound **12**, we can assign the value 77.87 ppm to the C1‘’ carbon, 76.87 ppm to the C1' carbon, and 135.43 ppm to C5.

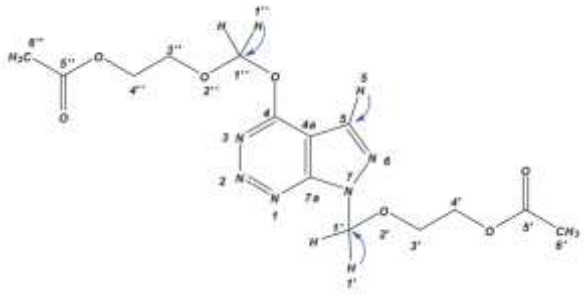
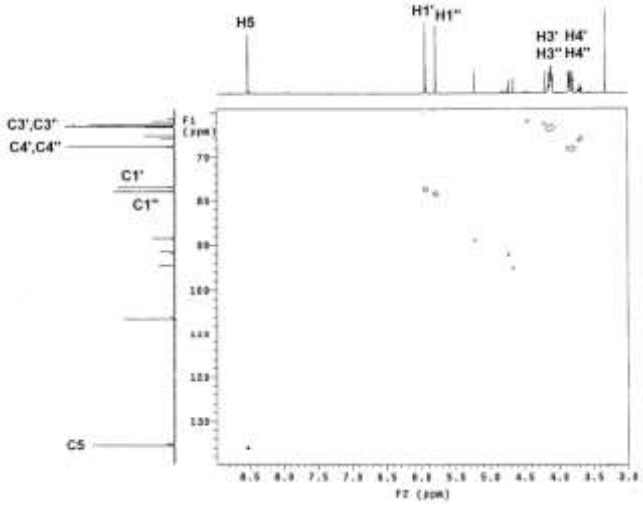
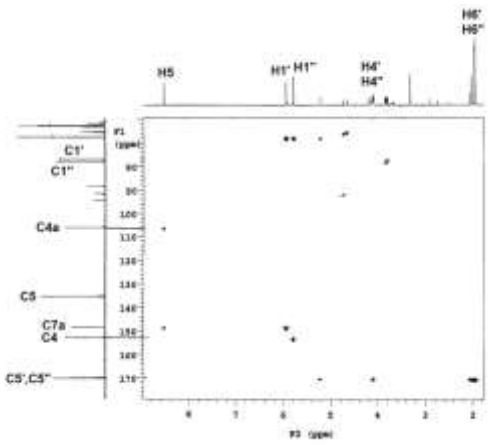


Figure 7: Illustration of the HMQC coupling of compound **12**



Spectrum 3: HMQC of product **12**

Analysis of the HMBC spectrum of compound **12** shows the absence of coupling between the protons of the methylene group (N-CH_2-O) (δ: 5.94, 77.87 ppm) and carbon C, which appears at 135.43 ppm (Spectrum 4). This observation suggests that alkylation took place on the nitrogen in position 7 and not on the nitrogen in position 6.



Spectrum 4: HMBC of product **12**

4. Conclusion

In conclusion, this study focused on the synthesis of acyclonucleosides of pyrazolo[3,4-d][1,2,3]triazines utilizing solid-liquid phase transfer catalysis. Through this method, we successfully synthesized a range of novel compounds, expanding the repertoire of accessible acyclonucleosides.

To ensure the structural integrity and confirm the identities of the synthesized products, we employed a comprehensive array of spectral analysis techniques. Notably, we utilized ^{1}H-NMR and ^{13}C-NMR spectroscopy to elucidate the proton and carbon environments within the molecules, respectively. Additionally, the application of advanced 2D-NMR techniques, including HMQC (Heteronuclear Multiple Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation), allowed us to establish correlations between different atoms in the molecules, facilitating the assignment of complex structural motifs.

Furthermore, mass spectrometry played a crucial role in confirming the molecular weights and fragmentation patterns of the synthesized compounds, providing further evidence to support their structural characterization.

Through the combination of these spectral methods, we have confidently identified and characterized the structures of the synthesized acyclonucleosides of pyrazolo[3,4-d][1,2,3]triazines. This work not only contributes to the expansion of chemical space in nucleoside chemistry but also underscores the importance of employing diverse analytical techniques for comprehensive structural elucidation in organic synthesis.

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