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**INVESTIGATION OF NEW DIHYDROPYRIDINE DERIVATIVE SOLUTIONS
BY USING OF 2D DOSY NMR****A.M.Maharramov, F.N.Naghiyev, I.G.Mamedov***Baku State University**bsu.nmrlab@mail.ru*

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As a result of the multicomponent condensation reaction between the thiophenylidenemalononitril and malononitriles with (S)-(-)-1-phenylethylamine new heterocyclic dihydropyridine derivative [(S)-(-)-6-amino-2-imino-1-(1-phenylethyl)-4-(thiophen-2-yl)-1,2-dihydropyridine-3,5-dicarbonitrile] was synthesized. DMSO- d_6 solutions of the new compound have been investigated in the presence of β -cyclodextrin by using the 2D DOSY method. As confirmed by our investigations, water molecules formed the combined intermolecular hydrogen bond with the β -cyclodextrin and sample. Also, the hydrodynamic radius of new dihydropyridine derivative has been calculated.

Keywords: dihydropyridine, cyclodextrin, hydrodynamic radius, DOSY NMR.

Introduction

Heterocyclic chemistry continues to be at the forefront of organic chemistry due to its importance across a variety of disciplines such as medicinal chemistry, biochemistry and materials science [1, 2]. Pyridine ring, a privileged structural core in heterocyclic chemistry, is present in many natural products such as nicotinic acid, nicotinamide and vitamin B_6 which play key roles in metabolism. In addition, functionalized pyridines have been shown to exhibit a broad range of biological activities including antimicrobial, antiulcer, anticancer, antipyretic and anti-inflammatory activities [3–7].

As known in the literature, cyclodextrins (CD), the basket-like cyclic oligosaccharides consisting of 6 or more α -D-glucopyranoside units, have the cavity for inclusion and are of great interest in the food industry, medicine, pharmaceutical, agriculture etc. These types of cavities make CD as most suitable hosts for a number of molecules in a variety of applications [8–13].

As well, NMR has important role at investigations of the formation of intermolecular complexes, association, self-organization, molecular recognition, reorientation and rearrangements of supramolecular structures under the action of various factors [14–29]. Beside, NMR spectroscopy and its experimental equipment enable measurements of the self-diffusion coefficients (SDC) in the high-resolution mode, which

in this case DOSY an important experimental method suitable for separation of the spectral contributions of different components of a system under study based on different sizes of the components. Moreover, having evaluated the size of the molecular system, one can assess its packing, degree of association, etc. [30–39].

Considering the above-indicated, we have investigated solutions of the new heterocyclic compound by using of NMR at the presence of β -CD.

Synthesis

All commercially available initial chemicals were obtained from Merck and Fluka companies and used without further purification. The β -CDs was obtained commercially from Sigma (purity, 98%).

The synthesis of (S)-(-)-6-amino-2-imino-1-(1-phenylethyl)-4-(thiophen-2-yl)-1,2-dihydropyridine-3,5-dicarbonitrile: thiophenylidenemalononitrile (4.5 mmol), malononitrile (4.5 mmol) and (S)-(-)-1-phenylethylamine (4.6 mmol) stirred 48 hours at room temperature, in 35 ml of methyl alcohol. The progress of the reaction was shown by thin-layer chromatography (TLC) (EtOAc/*n*-hexane, 3:1). The white powder was precipitated after evaporation of solvent, filtered by paper, re-crystallized from ethanol-water mixture and obtained in pure, yield – 91.7%, m.p. -189°C .

^1H NMR (300 MHz, $\text{DMSO}-d_6$): 1.55 (d, 3H, CH_3 , $^3J_{\text{H-H}} = 6.9$ Hz), 5.44 (k, 1H, CH-Ar, $^3J_{\text{H-H}} = 7.2$ Hz), 7.20-7.87 (m, 11H, 8Ar-H+NH₂+NH=); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): 21.70 (CH_3), 50.00 (CH-Ar), 79.77 ($=\text{C}_{\text{quat.}}$), 80.92 ($=\text{C}_{\text{quat.}}$), 116.85 (CN), 116.98 (CN), 127.14 ($2\text{CH}_{\text{arom.}}$), 127.21 ($\text{CH}_{\text{arom.}}$), 128.10 ($\text{CH}_{\text{thioph.}}$), 128.62 ($2\text{CH}_{\text{arom.}}$), 130.13 ($\text{CH}_{\text{thioph.}}$), 130.77 ($\text{CH}_{\text{thioph.}}$), 134.53 ($\text{C}_{\text{ar.}}$), 144.53 ($\text{C}_{\text{thioph.}}$), 152.29 ($=\text{C}_{\text{quat.}}$), 158.71 ($\text{N}=\text{C}_{\text{quat.}}$), 161.38 ($=\text{C}_{\text{quat.}}$); MS (ESI): 345.40, 241.3, 225.2 $[\text{M}+\text{H}]^+$.

Results and discussion

According to the concise literature survey above, substituted pyridines can be synthesized in a reaction between acetoacetanilides, aromatic aldehydes, malononitrile under different reaction conditions.

Herewith, we report for the first time the isolation and characterization of three cyclic dihydropyridine derivative via the reaction of thiophenylidenemalononitril and malononitriles with (S)-(-)-1-phenylethylamine at room temperature in methanol.

After determination of structure of synthesized (S)-(-)-6-amino-2-imino-1-(1-phenylethyl)-4-(thiophen-2-yl)-1,2-dihydropyridine-3,5-dicarbonitrile, its $\text{DMSO}-d_6$ solutions investigated in the presence of β -CD by using of 2D DOSY and obtained data were given in the table.

Diffusion coefficients $D \cdot 10^{-10}$ (m^2/s) of β -CD (5 mmol) and synthesized compound (5 mmol) in the 1 ml $\text{DMSO}-d_6$ solutions

β -CD+sample mixture	β -CD	Sample
	5.33	8.04
	1.43	2.21

As seen from the table the value of the diffusion coefficient in the β -CD + sample mixture significantly decreased. It demonstrates the

formation of any type of interaction between the β -CD and compound. For the confirmation of the type of interaction there were performed 2D NOESY and 2D ROESY spectra for the mixture. On the basis of ROESY/NOESY data, we can note that, formation of the inclusion complex between the investigated molecule and β -CD didn't occur. On the basis obtained NMR data revealed, that decreasing of diffusion coefficient connected by the presence of hydrogen bond between the water (residual solvent and hygroscopic sample water) and investigated compounds. The water molecules are formed the combined intermolecular hydrogen bond with the β -CD and sample.

From the known hydrodynamic radius of ferrocene (0.3 nm)⁴⁰, the hydrodynamic radius of the investigated compound was calculated using the equation (the diffusion coefficients of ferrocene (5 mmol) and sample (5 mmol) were $2.13 \cdot 10^{-9}$ and $1.29 \cdot 10^{-9} \text{ m}^2/\text{s}$ accordingly).

$$R = (D_{\text{reference}}/D_{\text{sample}}) \cdot R_{\text{reference}}$$

The calculated value of hydrodynamic radius of (S)-(-)-6-amino-2-imino-1-(1-phenylethyl)-4-(thiophen-2-yl)-1,2-dihydropyridine-3,5-dicarbonitrile equal to 0.495 nm .

We would like note that presented reaction conditions have never been tested before and the demonstrated three heterocyclic compound is new.

Experimental

The structure of synthesized compound confirmed by the 1D/2D NMR and MS methods.

NMR experiments have been performed on a Bruker AV III FT NMR spectrometer AVANCE 300 (300 MHz for ^1H and 75 MHz for ^{13}C) with a BVT 3200 variable temperature unit in 5 mm sample tubes using Bruker Stand-

ard software (TopSpin 3.1). The ^1H and ^{13}C chemical shifts were referenced to internal tetramethylsilane (TMS); the experimental parameters for ^1H : digital resolution = 0.23 Hz, SWH = 7530 Hz, TD = 32 K, SI = 16 K, 90° pulse-length = 10 μs , PL1 = 3 dB, ns = 1, ds = 0, d1 = 1 s; for ^{13}C : digital resolution = 0.27 Hz, SWH = 17985 Hz, TD = 64 K, SI = 32 K, 90° pulse-length = 9 μs , PL1 = 1.5 dB, ns = 100, ds = 2, d1 = 3 s. ^{13}C DEPT 90 and 135, HMQC, HMBC NMR spectra were performed by the Bruker Standard software.

DOSY NMR experiments were carried out using a Bruker AV III FT NMR spectrometer equipped with a pulsed-gradient unit. The operating frequency was 300 MHz with a diffusion time of 100 ms and the gradient pulse of 3000 ms. The pulsed-gradient unit produced magnetic field gradients of 54 G cm^{-1} in the z-direction. NMR-grade DMSO- d_6 (99.7 %, containing 0.3% H_2O) was used. The pulse sequence used was a bipolar pulse longitudinal eddy current delay (BPLED) sequence. The gradient strength (g) was increased from 2 to 95% of the maximum in a quadratic ramp.

Electrospray mass spectra of synthesized compound were run with an ion-trap instrument (Esquire 6000 Ion Trap Mass Spectrometer) equipped with an electrospray (ESI) ion source. For electrospray ionization, the drying gas and flow rate were optimized according to the particular sample with 35 p.s.i. nebulizer pressure. Scanning was performed from m/z 100 to 1200 in methanol solution. The compounds were observed in the positive mode (capillary voltage = 80–105 V).

The purity of the synthesized compounds were confirmed by TLC on commercial aluminum-backed plates of silica gel (60 F254), iodine vapor was used as visualizing agent, eluent – 5:2 hexane/ethyl acetate.

Melting points were measured on an Electrothermal 9100 apparatus without correction.

Conclusions

We have successfully synthesized a new heterocyclic dihydropyridine derivative via the reaction of thiophenylidenemalononitril and

malononitriles with (S)-(–)-1-phenylethylamine at room temperature in methanol, i.e., under conditions that have never been tested before. This method represents a new efficient synthesis route to other functionalized cyclic dihydropyridines. Also, we investigated DMSO- d_6 solutions of the new compound in the presence of β -CD. As confirmed by our investigations, water molecules formed the combined intermolecular hydrogen bond with the β -CD and sample.

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YENİ DİHİDROPIRIDİN TÖRƏMƏSİ MƏHLULLARININ 2D DOSY NMR METODU İLƏ TƏDQIQI

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Tiofenilidenmalononitril, malononitril və (S)-(–)-1-feniletilaminin çoxkomponentli kondensləşmə reaksiyasından yeni heterotsiklik dihidropiridin törəməsi sintez olunmuşdur. Yeni dihidropiridin törəməsinin DMSO- d_6 məhlulları β -tsiklodekstrin iştirakında diffusiya spektroskopiyasının köməyiylə tədqiq edilmişdir. Tədqiqatlar nəticəsində müəyyən olunmuşdur ki, su molekulu nümunə və β -tsiklodekstrin molekulları arasında kombinə olunmuş hidrogen rabitəsi əmələ gətirir. Eləcə də dihidropiridin törəməsinin hidrodinamik radiusu hesablanmışdır.

Açar sözlər: dihidropiridin, tsiklodekstrin, hidrodinamik radius, DOSY NMR.

ИССЛЕДОВАНИЕ РАСТВОРОВ НОВОГО ПРОИЗВОДНОГО ДИГИДРОПИРИДИНА С ИСПОЛЬЗОВАНИЕМ 2D DOSY ЯМР

A.M.Магєррамов, Ф.Н.Нагєев, И.Г.Мамєдов

Синтезировано новое гетероциклическое производное дигидропиридина в результате многокомпонентной реакции конденсации между тиофенилиденмалононитрилом и малононитрилами с (S)-(–)-1-фенилэтиламином. Исследованы растворы DMSO- d_6 нового соединения в присутствии β -циклодекстрина с использованием метода диффузионной спектроскопии и подтверждено, что молекулы воды образуют комбинированную межмолекулярную водородную связь с β -циклодекстрином и образцом. Также был рассчитан гидродинамический радиус нового производного дигидропиридина.

Ключевые слова: дигидропиридин, циклодекстрин, гидродинамический радиус, DOSY ЯМР.