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SYNTHESIS OF 6'-CARBAMOYLMETHYLTHIO-5'-CYANO-1',4'-DIHYDRO-3,4'-BIPYRIDINE-3'-CARBOXYLIC AND 6'-CARBAMOYLMETHYLTHIO-5'-CYANO-1',4'-DIHYDRO-4,4'-BIPYRIDINE-3'-CARBOXYLIC ESTERS AND INVESTIGATION OF THEIR STABILITY. 2*. ESTERS OF 6'-CARBAMOYLMETHYL-THIO-5'-CYANO-1',4'-DIHYDRO-4,4'-BIPYRIDINE-3-CARBOXYLIC ACIDS

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The stability of solutions of esters of 6'-carbamoylmethylthio-5'-cyano-2'-methyl-1',4'-dihydro-4,4'-bipyridine-3'-carboxylic acids was investigated by HPLC. The corresponding esters of 6'-carbamoylmethylthio-5'-cyano-4,4'-bipyridine-3'-carboxylic acids, esters of 8-cyano-5-methyl-3-oxo-7-(4-pyridyl)-2,3-dihydro-7H-thiazolo-[3,2-a]pyridine-6-carboxylic acids, methyl 3-amino-2-carbamoyl-6-methyl-4-(4-pyridyl)-4,7-dihydrothieno[2,3-b]pyridine-5-carboxylate, and methyl 3-amino-2-carbamoyl-6-methyl-4-(4-pyridyl)-thieno[2,3-b]pyridine-5-carboxylate were synthesized as standard compounds (typical impurities). Analysis by HPLC was realized under the conditions of reverse-phase chromatography. It was established that solutions of the investigated compounds (with mixtures of acetonitrile with phosphate buffer, having pH values of not less than 3 and not more than 5, as solvents) are stable for one month when the solutions are stored in a place protected against light. It is also necessary to use chromatographic systems in which the aqueous components have pH 3-5 during determination of the purity of the esters of 6'-carbamoylmethylthio-5'-cyano-2'-methyl-1',4'-dihydro-4,4'-bipyridine-3'-carboxylic acid by HPLC in order to separate the analyzed sorbates and their typical impurities more completely.

Keywords: 4,4′-bipyridines, 2,3-dihydro-7H-thiazolo[3,2-*a*]pyridines, thieno[2,3-*b*]pyridines, HPLC.

Earlier [2] we synthesized a series of esters of 6'-carbamoylmethylthio-5'-cyano-1',4'-dihydro-4,4'-bipyridine-3'-carboxylic acids, which being regioisomers of the cardiotonic preparation milrinone [3] are of interest as potential positively acting inotropic compounds. However, it is known [2] that hydrogenated 2-alkylthio-4,4'-bipyridines are unstable in acidic solutions.

In the present work we investigated the stability of the above-mentioned esters in mixtures of acetonitrile and a 0.1% solution of phosphoric acid (solutions with pH 2.3, Table 1) and an acetonitrile–phosphate buffer (solutions with pH 3-9, Table 1).

*For Communication 1, see [1].

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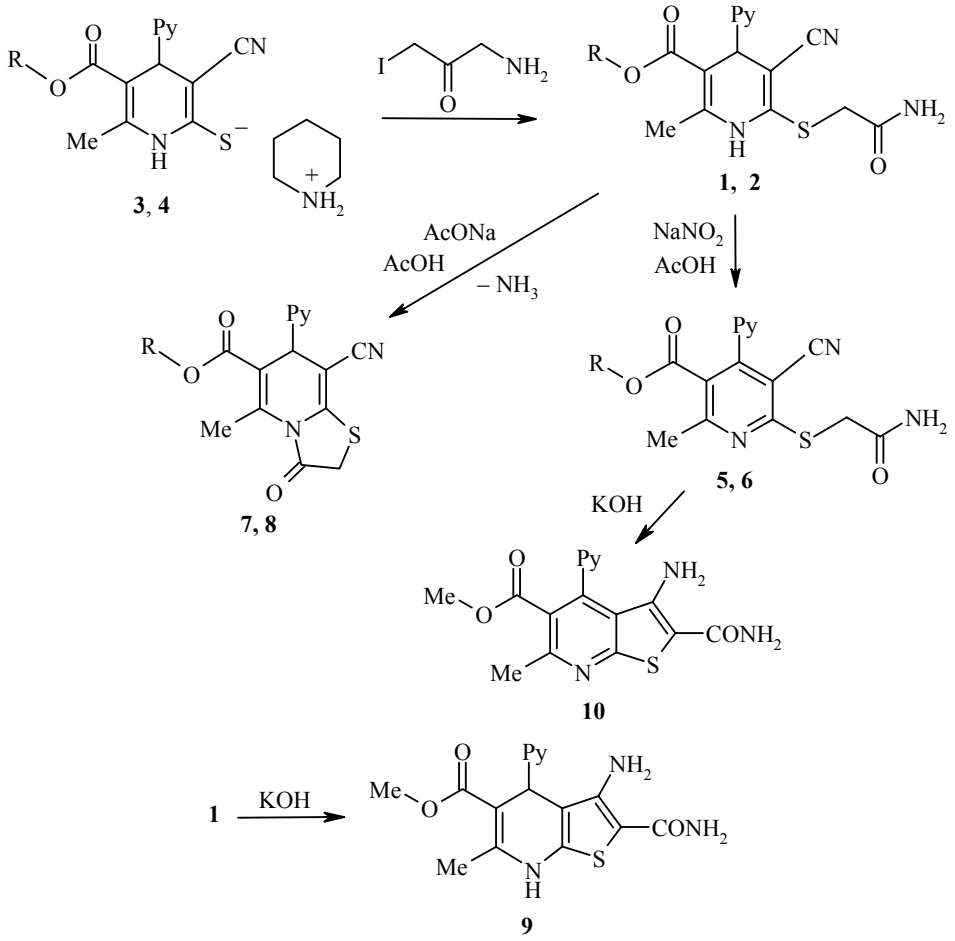
Earlier [1] we studied the stability of esters of 6'-carbamoylmethylthio-5'-cyano-1',4'-dihydro-3,4'-bipyridine-3'-carboxylic acids and showed that solutions of the investigated compounds in a mixture of acetonitrile with phosphate buffer (pH 3-5) were stable for one month when the solutions were stored in a place protected from light. Storage of the solutions in a place not protected from light (irrespective of the pH) led to the formation of the corresponding esters of 6'-carbamoylmethylthio-5'-cyano-3,4'-bipyridine-3'-carboxylic acids. The presence of esters of 8-cyano-5-methyl(phenyl)-3-oxo-7-(3-pyridyl)-2,3-dihydro-7H-thiazolo-[3,2-*a*]pyridine-6-carboxylic acids (not more than 4%) was only detected in acetonitrile–phosphoric acid solutions that had been stored in a place protected from light. A series of as yet unidentified products were found in the solutions with pH 7-9.



1-**10** Py = 4-(pyridine-4-yl); **1, 3, 5, 7** R = Me; **2**, **4**, **6, 8** R = Et

The subjects of the investigation in the present work esters of 6'-carbamoylmethylthio-5'-cyano-2'-methyl-1',4'-dihydro-4,4'-bipyridine-3'-carboxylic acids **1** [2] and **2** [4] were prepared by alkylation of the corresponding 3′-alkoxycarbonyl-5'-cyano-2'-methyl-1',4'-dihydro-4,4'-bipyridine-6′-thiolates **3** and **4** [5] with iodoacetamide. As standard compounds (the supposed transformation products during storage of solutions of the investigated esters) for study of the stability of the solutions of the esters **1** and **2** we used the corresponding esters of 6'-carbamoylmethylthio-5'-cyano-2'-methyl-4,4'-bipyridine-3'-carboxylic acids **5** [2] and **6**, esters of 8-cyano-5-methyl-3-oxo-7-(4-pyridyl)-2,3-dihydro-7H-thiazolo[3,2-*a*]pyridine-6-carboxylic acids **7** [6] and **8**, methyl 3-amino-2-carbamoyl-6-methyl-4-(4-pyridyl)-4,7-dihydrothieno[2,3-*b*]pyridine-5-carboxylate **9** [2], and methyl 3-amino-2-carbamoyl-6-methyl-4-(4-pyridyl)thieno[2,3-*b*]pyridine-5-carboxylate **10** [2].

TABLE 1. Investigation of the Stability of Solutions of Compounds **1** and **2** by Reverse-Phase HPLC (Gradient Regime)*

Compound	Content of investigated compounds in analyzed solutions, %*[2], at					
	pH 2.3		pH 3	pH 5	pH 7	pH 9
	Just after dissolution	After one month (in the dark)				
1	98.7	91.5	98.5	98.5	90.5	45
5+7	0.8	4.1	1	1	1	—
1A = 9	—	—	—	—	8	50
1C = 10	—	—	—	—	—	4
1X*[3]	0.5	4.4	0.5	0.5	0.5	1
2	98.7	91	98.4	98.4	93.5	81
6+8	1	4.7	1.3	1.3	1.3	–
2A	—	—	—	—	5	17
2C	—	—	—	—	—	1.7
2X*[3]	0.3	4.3	0.3	0.3	0.3	0.3

* The conditions for chromatographic analysis are given in the experimental section.

*[2] Quantitative analysis was performed by normalization of the areas (λ = 254 nm) [7]. Solutions with pH 2.3: 5% acetonitrile in 0.3% solution of phosphoric acid in water; pH 3: acetonitrile–0.01 M phosphate buffer pH 3 (25:75); pH 5: acetonitrile–0.01 M phosphate buffer pH 5 (25:75); pH 7: acetonitrile–0.01 M phosphate buffer pH 7 (30:70); pH 9: acetonitrile–0.01 M phosphate buffer pH 9 (30:70).

*[3] The total content of unidentified impurities (number of unidentified impurities not more than 3).

The bipyridines **5** and **6** were obtained by the action of sodium nitrite on the corresponding 1',4'-dihydro-4,4′-bipyridines **1** and **2** in boiling acetic acid. The 2,3-dihydro-7H-thiazolo[3,2-*a*]pyridines **7** and **8** were obtained by boiling compounds **1** and **2** in acetic acid with the addition of sodium acetate. 4,7-Dihydrothieno[2,3-*b*]pyridine **9** or thieno[2,3-*b*]pyridine was obtained by briefly heating a solution of compound **1** or **5** respectively with a catalytic amount of potassium hydroxide.

The same gradient reverse-phase regime of HPLC as in [1] was used to investigate the stability of the solutions of compounds **1** and **2** [1]. However, it was established that the given chromatographic system does not make it possible to distinguish between such pairs of compounds as **5**/**7** and **6**/**8** [retention times, t_r: 6.65 (**5**), 6.64 (**7**), and 7.45 (**6**), 7.49 min (**8**)]. The data for these compounds presented in Table 1 must therefore be regarded as the total contents **5** + **7** and **6** + **8**.

If the sample is introduced immediately after the compounds **1** and **2** have dissolved in the mobile phase (solutions pH 2-3, Table 1), the peaks corresponding to the dihydrobipyridines **1** and **2** (the content of the latter in the solutions being analyzed is 98.7%) predominate on the obtained chromatograms. On the chromatograms of the same samples after one month the areas of the peaks corresponding to compounds **1** and **2** are reduced to 91-91.5%. At the same time there is an increase in the areas of the peaks corresponding to **5** + **7** and **6** + **8** (from 0.8 to 4.1% for solution **1**, from 1 to 4.7% for solution **2**). According to data obtained by means of a spectrophotometric detector on a *ProStar 330* diode matrix, immediately after dissolution of compounds **1** and **2** in the mobile phase the UV spectra of the peaks coinciding with the standards **5**-**8** in retention time coincide with the UV spectra of the model compounds **5** and **6**, while after one month they coincide with the UV spectra

of model compounds **7** and **8**. This does not contradict the data we obtained earlier for esters of 6'-carbamoylmethylthio-5'-cyano-1',4'-dihydro-3,4'-bipyridine-3'-carboxylic acids. As demonstrated in [1], after one month during storage of the solutions of the compounds with pH 2.3 in the dark the corresponding esters of 8-cyano-5-methyl(phenyl)-3-oxo-7-(3'-pyridyl)-2,3-dihydro-7H-thiazolo[3,2-*a*]pyridine-8-carboxylic acids were formed.

According to the data in Table 1, on the chromatograms of the solutions of compounds **1** and **2** with pH 3 and pH 5 there is only a slight increase in the area of the peaks corresponding to compounds **5** + **7** and **6** + **8** (not more than 0.3%), i.e., the solutions are stable for one month when stored in a place protected from light. It was established (these data are not included in Table 1, since they are identical with the results obtained in [1]) that oxidation of the dihydrobipyridines **1** and **2** to the corresponding bipyridines **5** and **6** takes place under the influence of light in all the investigated solutions irrespective of the pH value. Analysis of the solutions with pH 7 one month after dissolution of the investigated compounds showed a decrease in the content of the dihydrobipyridines **1** and **2** (from 98.7 to 90.5% for solution of **1**, from 98.7 to 93.5% for solution **2**) and the formation of products with characteristics close to those of the compounds of type **A** (content of **1A** 8%, **2A** 5%) unidentified in [1]. In solutions with pH 9 after one month the content of the dihydrobipyridines **1** and **2** amounts to only 81 for solution of **2** (R = Et) and 45% for solution of **1** (R = Me). At the same time the content of compound **2A** amounts to 17, while that of **1A** amounts to 50%. In addition the formation of products close to the unidentified compounds **C** [1] is observed (content of compound **1C** 4, **2C** 1.7%). Comparison of compounds **1A** and **1C** with the model compounds **9** and **10** showed that compound **1A** is identical with compound **9** both in retention time and in UV spectrum while the model compound **10** corresponds to compound **1C**. This can be explained by the fact that an intramolecular Thorpe cyclization of **1**, leading to the dihydrothienopyridine **9**, takes place in the solution with pH 7, while oxidation of **1** and intramolecular cyclization or intramolecular cyclization of **1** and oxidation with the formation of the thienopyridine **10** take place in the solution with pH 9.

Table 2 shows the dependence of the retention time of compounds **1**, **2**, and **5**-**8** on the pH of the phosphate buffer and the concentration of acetonitrile in the mobile phase (an isocratic regime of HPLC). As expected, in reverse-phase chromatography the retention time of the sorbate decreases with increase in the concentration of acetonitrile. It is seen that there is a significant change in the retention time of the bipyridines **5** and **6** with change in the pH of the mobile phase from 3 to 5.

The main disadvantage of the described gradient regime (Table 1) was the unsatisfactory separation of the pairs of compounds **7**/**5** and **8**/**6**. On the basis of the data in Table 2 the selectivity or the relative retention (α) was calculated for the following pairs of compounds: **5**/**1**, **7**/**1**, **7**/**5**, **6**/**2**, **8**/**2**, **8**/**6**. It is known that good

TABLE 2. The Dependence of the Retention Time of Compounds **1**, **2**, and **5**-**8** on the pH of the Phosphate Buffer and the Concentration of Acetonitrile in the Mobile Phase (Isocratic Regime)*

Compound	Retention time, t_r, min, at									
	pH 3				pH 5		pH 7		pH 9	
	20%	25%	30%	40%	25%	35%	30%	35%	30%	40%
1	15.33	5.65	3.33	1.93	4.13	2.34	3.32	2.85	3.45	2.20
5	—	33.49	14.82	3.56	9.27	3.38	3.70	3.09	3.83	2.36
7	—	25.30	14.84	4.99	20.55	7.07	9.52	7.08	9.80	4.39
2	41.25	13.39	6.44	2.41	7.71	3.18	5.25	3.73	5.13	2.75
6	—	—	31.15	5.16	15.38	4.62	5.61	4.06	5.45	2.92
8	—	—	35.65	8.49	42.20	11.91	17.18	11.10	16.71	6.27

* For the conditions of chromatographic analysis, see the experimental section.

chromatographic separation of two substances is possible with selectivity $\alpha > 1.5$. It follows from the data presented in Table 3 that the chromatographic system in which the mobile phase has pH 3-5 meets this requirement. If the chromatographic system in which the aqueous component of the mobile phase has pH > 5 is used, a problem can arise in the separation of the dihydrobipyridines **1** and **2** with the corresponding bipyridines **5** and **6** ($\alpha < 1.4$). It is seen that the selectivity of separation of the pairs of compounds **7**/**5** and **8**/**6** deteriorates in more acidic systems. It is not therefore surprising that the separation of the bipyridines **5** and **6** with the corresponding thiazolopyridines **7** and **8** is unsatisfactory ($\alpha < 1.1$) under the conditions of the gradient regime, where a 0.1% solution of phosphoric acid (pH 2.3) was used as aqueous component of the mobile phase.

An isocratic chromatographic system with a mobile phase consisting of 35% acetonitrile and 65% of a 0.01 M phosphate buffer (pH 5) was used to confirm the formation of the thiazolopyridines **7** and **8** in the solutions of compounds **1** and **2** with pH 2.3 that had been stored in the dark for one month. It was established that immediately after dissolution of compounds **1** and **2** in the mobile phase apart from the dihydrobipyridine **1** or **2** the chromatogram only contained the respective bipyridine **5** or **6**, while analysis of the solutions (pH 2.3) of compounds **1** and **2** that had been stored in the dark for one month showed that the content of the bipyridine **5** or **6** had not changed, whereas the corresponding thiazolopyridine **7** or **8** had formed.

Thus, the chromatographic behavior of compounds **1**, **2**, and **5-8** under various conditions of reverse-phase chromatography was studied in the present work. It was established that the bipyridines **5** and **6** are formed under the influence of light in all the investigated solutions irrespective of the pH value. It was shown that the formation of thiazolopyridines **7** and **8** is observed in the solutions with pH < 3 when the solutions are stored in the dark, while the formation of the corresponding 4,7-dihydrothieno[2,3-*b*]pyridines **A** and/or thieno[2,3-*b*]pyridines **C** is observed in the solutions with pH > 5. It was established that solutions of the investigated compounds (when mixtures of acetonitrile with the phosphate buffer with pH not less than 3 and not greater than 5 are used as solvents) are stable for one month provided that the solutions are stored in a place protected from light. Moreover, it is also necessary to use chromatographic systems in which the aqueous component of the mobile phase has pH 3-5 during determination of the purity of the dihydrobipyridines **1** and **2** by HPLC (determination of the content of the typical impurities **5-8**) in order to obtain more complete separation of the sorbates being analyzed.

TABLE 3. The Selectivity of the Chromatographic System* in Relation to the pH of the Mobile Phase

Two separated compounds	Selectivity*[2], α			
	AN – PB (40 : 60), pH 3	AN – PB (35 : 65), pH 5	AN – PB (35 : 65), pH 7	AN – PB (40 : 60), pH 9
5/1	5.33	2.34	1.18	1.24
7/1	9.17	6.70	4.26	4.41
7/5	1.72	3.02	3.60	3.55
6/2	4.28	1.88	1.37	1.15
8/2	8.22	6.38	5.28	3.97
8/6	1.92	3.38	3.83	3.47

* Isocratic regime. For the conditions of chromatographic analysis, see the experimental section.

*[2] The selectivity or the relative retention of the separated compounds: $\alpha_{ij} = t_{ri} - t_0/t_{rj} - t_0 = k_i/k_j$, where t_{ri} and t_{rj} are the retention times of the corresponding *i*-th and *j*-th components of the separated pair;
t_0 − {uracil} = 1.56 min.

EXPERIMENTAL

The IR spectra were recorded in vaseline oil on a Perkin-Elmer 580B instrument. The ^{1}H NMR spectra were recorded on a WH 90/DC spectrometer (90 MHz) with HMDS as internal standard (δ 0.05 ppm). The reactions and the individuality of the substances were monitored by TLC on Silufol UV-254 plates with 2:1:5 chloroform–hexane–acetone as eluent. The compounds were recrystallized from ethanol.

The synthesis and the characteristics of compounds **1**, **5**, **9**, and **10** were described in [2], compound **2** in [4], and compound **7** in [6].

Ethyl 6'-Carbamoylmethylthio-5'-cyano-2'-methyl-4,4'-bipyridine-3'-carboxylate (6). A solution of ethyl 6'-carbamoylmethylthio-5'-cyano-2'-methyl-1',4'-dihydro-4,4'-bipyridine-3'-carboxylate (**2**) (0.36 g, 1 mmol) in acetic acid (3 ml) was heated to boiling, and sodium nitrite (0.11 g, 1.5 mmol) was added. When the release of NO_2 had stopped the reaction mixture was poured into water (10 ml) and neutralized with ammonia. The precipitate was filtered off and washed with water (10 ml). We obtained 0.20 g (58%) of compound **6**; mp 162-164°C. IR spectrum, ν, cm^{-1}: 1684, 1728 (C=0); 2224 (C≡N); 3164, 3312 (NH_2). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 0.95 and 4.03 (5H, t and q, *J* = 7, OC_2H_5); 2.68 (3H, s, 6-CH_3); 3.97 (2H, s, SCH_2); 6.06 and 6.53 (2H, br. s and br. s, $CONH_2$); 7.32 and 8.30 (4H, dd and dd, *J* = 5, C_5H_4N). Found, %: C 57.26; H 4.28; N 15.86; S 9.00. $C_{17}H_{16}N_4O_3S$. Calculated, %: C 57.29; H 4.53; N 15.72; S 9.00.

Ethyl 8-Cyano-5-methyl-3-oxo-7-(4'-pyridyl)-2,3-dihydro-7H-thiazolo[3,2-*a*]pyridine-8-carboxylate (8). A mixture of the ester **2** (0.36 g, 1 mmol) and sodium acetate (0.02 g, 0.25 mmol) in acetic acid (3 ml) was heated for 3 h. After cooling ethanol (5 ml) was added to the reaction mixture. The mixture was then neutralized with ammonia, and the precipitate was filtered off and washed with water (5 ml). The filtrate was poured into water (10 ml), and the precipitate was filtered off and recrystallized from ethanol. We obtained 0.18 g (53%) of compound **8**; mp 162-164°C. IR spectrum, ν, cm^{-1}: 1667, 1718 (C=0); 2202 (C≡N). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.12 and 4.07 (5H, t and q, *J* = 7, OC_2H_5); 2.68 (3H, s, 6-CH_3); 3.94 (2H, s, 2-CH_2); 4.72 (1H, s, 7-H); 7.16 and 8.60 (4H, dd and dd, C_5H_4N). Found, %: C 59.58; H 4.23; N 12.30; S 9.24. $C_{17}H_{15}N_3O_3S$. Calculated, %: C 59.81; H 4.43; N 12.31; S 9.39.

Conditions of HPLC. Gradient Regime. The measurements were made on a Varian ProStar 240 liquid chromatograph consisting of a ProStar 240 gradient pump, a spectrophotometric detector based on a ProStar 330 diode matrix (λ = 254 nm), and a ProStar 410 autosampler. The Agilent column (150×4.6 mm) was packed with Zorbax SB-C18. The mobile phase was acetonitrile–0.1% phosphoric acid solution in water (pH 2.3) with linear gradient (15 min) from 5 to 95% acetonitrile. The consumption rate of the mobile phase was 1.0 ml/min. The samples (10 μl, in the mobile phase 0.5 mg/ml) were introduced by means of the autosampler. Retention times, t_r: 5.54 (**1**); 6.65 (**5**); 6.64 (**7**); 5.28 (**1A** = **9**); 6.70 (**1C** = **10**); 6.11 (**2**); 7.45 (**6**); 7.49 (**8**); 5.95 (**2A**); 7.52 min (**2C**). The retention time of the conditionally unsorbed substance (uracil) was t_0 1.55 min.

The preparation of the analyzed solutions was described in [1].

Isocratic Regime. The measurements were made on a chromatographic system consisting of a Waters 510 pump, a Du Pont Instruments UV Spectrophotometer detector (λ = 254 nm), and a Hewlett Packard HP 3395 integrator. The Agilent column (150×4.6 mm) was packed with Zorbax Extend-C18. The mobile phase was acetonitrile (AN)–phosphate buffer (PB). The solutions of 0.01 M PB (pH 3, 5, 7, 9) were prepared by titration of 0.01 M phosphoric acid with potassium hydroxide to the required pH value [1]. The consumption rate of the mobile phase was 1.0 ml/min. The samples (50 μl, in the mobile phase *c* = 0.05 mg/ml) were introduced without stopping the flow by means of a Rheodyne 7125 loop tap dispenser. For the ratio of the components of the mobile phase and the retention time, see Table 2.

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