SYNTHESIS AND CHARACTERIZATION OF DIALKYL ESTERS OF 1,2,4,5-TETRAZINE-3,6-DICARBOXYLIC ACID

Štěpán Frebort[a1], Numan Almonasy[a2,*], Radim Hrdina[a3], Antonín Lyčka[b], Miroslav Lísa[c1] and Michal Holčapek[c2]

[a] *Department of Technology of Organic Compounds, Faculty of Chemical Technology, University of Pardubice, Studentská 95, CZ-532 10 Pardubice, Czech Republic; e-mail: [1] tramvajak.ostrava@post.cz, [2] numan.almonasy@upce.cz, [3] radim.hrdina@upce.cz*

[b] *Research Institute for Organic Syntheses, Rybitví 296, CZ-533 54 Rybitví, Czech Republic; e-mail: antonin.lycka@vuos.com*

[c] *Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 95, CZ-532 10 Pardubice, Czech Republic; e-mail: [1] miroslav.lisa@upce.cz, [2] michal.holcapek@upce.cz*

Received August 21, 2007
Accepted December 5, 2007

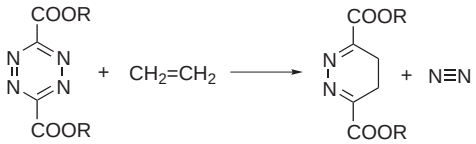
Synthesis and characterization of a series of dialkyl esters of 1,2,4,5-tetrazine-3,6-dicarboxylic acid are reported. These compounds were prepared by a two-stage synthesis: re-esterification of dimethyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate in the presence of aluminium triethoxide and subsequent dehydrogenation of dialkyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylates. The structures of the prepared compounds were confirmed by NMR and mass spectra.

Keywords: Tetrazines; Dihydrotetrazines; Esterification; Aluminium triethoxide; Oxidation.

1,2,4,5-Tetrazines have been widely investigated because of the possibility of a large variety of practical applications. Investigations have led to their applications as biocides[1–6], high-nitrogen energetic materials[7,8], and intermediates for syntheses of a variety of heterocyclic compounds[9–12]. Over the last twenty years, 3,6-disubstituted compounds of 1,2,4,5-tetrazine type **1** have been intensively studied[9–15]. Dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate is the best-known compound of this type from the viewpoint of application and key intermediate in inverse-type Diels–Alder reaction[9–15]. In the sense of this reaction, some dialkyl 1,2,4,5-tetrazine-3,6-dicarboxylates may have potential use for improving the shelf-life of fruits and vegetables by trapping the ethene gas generated by these crops[16]. Ethene rapidly reacts with 1,2,4,5-tetrazine compounds at room temperature and produces dihydropyridazine compounds with concomitant formation of nitrogen gas[17]

doi:10.1135/cccc20080107

(Scheme 1). A characteristic colour change is typical of this reaction: the bright-red reactants turned to yellow products.



1, R = alkyl

SCHEME 1

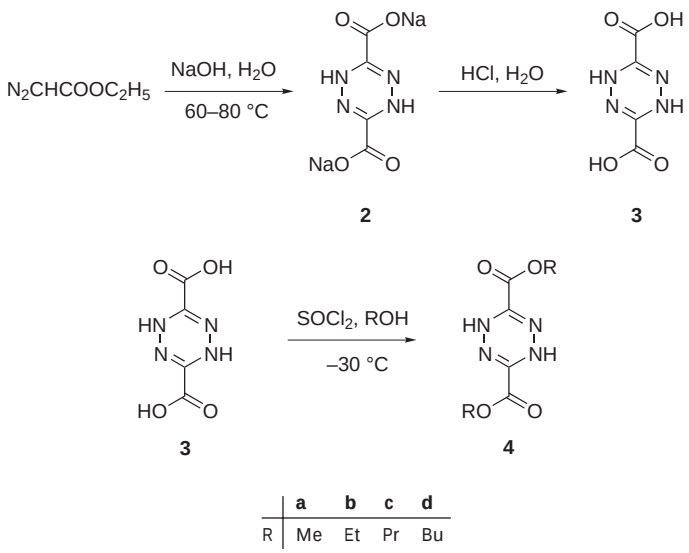
1,2,4,5-Tetrazines are unstable in the presence of water[18], which is an impediment in their applications in moist or humid conditions, as it is the case of fruits and vegetables. Therefore the tetrazines are incorporated in a hydrofobic ethene-permeable substrate which does not contain hydroxy groups. The used hydrophobic polymeric materials include siliconepolycarbonate, polystyrene, polyethylene and polypropylene[16]. The preferred tetrazine esters used as active substances are dioctyl, didecyl and dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate[16].

In spite of the wide range of applications mentioned above, these compounds have not received sufficient attention as far as their syntheses are concerned. Therefore, our initial studies were aimed at finding the optimum method for their preparation.

RESULTS AND DISCUSSION

Because of the instability of 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylic acid (**3**) in acid media at enhanced temperatures[11], we failed to get dialkyl esters by using the standard esterification method. Therefore, we used the procedure described by Boger[13] for synthesis of dimethyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**4a**). According to this method, the diester was prepared by the three-step reaction of ethyl diazoacetate with sodium hydroxide in water at 70 °C giving sodium 1,2,4,5-tetrazine-3,6-dicarboxylate (**2**), which was treated with aqueous HCl to give **3**. The following reaction of **3** with thionyl chloride and methanol at –30 °C then gave **4a** (Scheme 2).

The same procedure was adopted for preparation of diethyl, dipropyl, and dibutyl esters **4b**–**4d**, but their reaction yields were very low (15, 10 and 6% for **4b**, **4c** and **4d**, respectively). The reaction yields drastically decrease with increasing alkyl chain length. So far our efforts to prepare

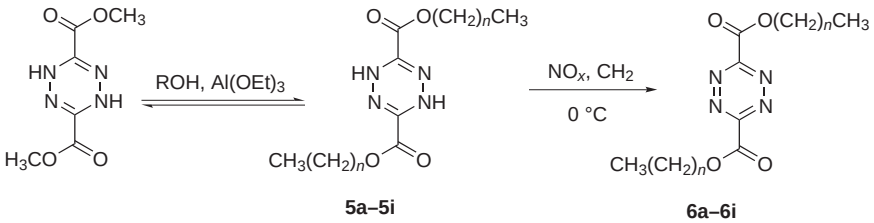


SCHEME 2

longer-chain esters using the same method have failed. Therefore, for the synthesis of these esters we have adopted a modification of the common procedure consisting in re-esterification of **4a** with various alcohols at boiling temperature in the presence of $Al(OC_2H_5)_3$ as a catalyst. In this way, a series of dialkyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylates **5a**–**5i** was prepared (Scheme 3). The equilibrium of this reaction is usually shifted towards the product by distilling off the methanol formed.

Generally, the re-esterification reactions of **4a** with long-chain alcohols were easy and without complications. However, with lower-boiling alcohols the re-esterification was difficult. Therefore, the re-esterification of **4a** with short-chain, e.g. ethyl, propyl and butyl, alcohols was performed in an autoclave. Our attempts at preparing such esters at normal atmospheric pressure led to very low yields and conversions. Usually, the products were present in a yield of ca. 50% together with the non-reacted starting material **4a**. The use of autoclave was successful: the reaction temperature increased, and hence also the yields and conversions. Nevertheless, the reaction yields and purity of products **5b**–**5d** were not as good as those of long-chain esters **5e**–**5i**. The reaction course and purity of products were checked by HPLC with acetonitrile–water (4:1) as the mobile phase.

The oxidation method adopted for all the dihydro compounds **4** was identical with the procedures described in the literature[13]. The synthesis is shown in Scheme 3. The prepared compounds were characterized by ^{1}H, ^{13}C, and ^{15}N NMR spectra, elemental analysis and mass spectra. The molecular weights (MW) of all the studied compounds were confirmed by the measurement of their atmospheric-pressure chemical ionization (APCI) mass spectra in both polarity modes. Positive-ion APCI mass spectra show mainly peaks of protonated molecules $[M + H]^+$, while the negative-ion APCI spectra provide complementary information with $[M - H]^-$ ions. In addition to these even-electron ions, typical of soft ionization mass spectra, the odd-electron molecular radical ions M^+ in the positive-ion mode and M^- in the negative-ion mode are observed as well, which is quite unusual in APCI [19]. The type of alkyl substituent can be identified on the basis of neutral losses of side chain, as reported earlier[20].



SCHEME 3

5,6	a	b	c	d	e	f	g	h	i
n	1	2	3	4	5	6	7	8	9

CONCLUSIONS

Aluminium triethoxide was used successfully as a catalyst for the re-esterification of dimethyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate with various alcohols. It was found that the re-esterification with longer-chain alcohols (C_5–C_{10}) is easier than that with shorter-chain alcohols (C_1–C_4). The corresponding dialkyl 1,2,4,5-tetrazine-3,6-dicarboxylates **6** were obtained by oxidation of the 1,4-dihydro compounds with nitrogen oxides (NO_x).

EXPERIMENTAL

The chemicals used were purchased from Fluka and their melting points were checked on a hot-stage microscope. The ^{1}H, ^{13}C and ^{15}N NMR spectra (δ, ppm; *J*, Hz) were recorded on a Bruker Avance 500 spectrometer operating at 500.13 MHz for ^{1}H, 125.76 MHz for ^{13}C and

50.68 MHz for ^{15}N. The samples were dissolved in deuteriochloroform and measured at room temperature. The ^{1}H and ^{13}C chemical shifts were referenced to internal TMS. The ^{15}N chemical shifts were referenced to external nitromethane in coaxial capillary (δ 0.0). The positive values of chemical shifts denote shifts of signals to higher frequencies with respect to the standard. The ^{13}C chemical shifts of COO groups were assigned having analysed proton-coupled ^{13}C NMR spectra in which these groups form broadened triplets due to $^{3}J(^{13}C,H)$ coupling constants from OCH_2 groups (in contrast to singlets of aromatic). Mass spectrometry analyses were performed on an Esquire 3000 ion trap analyzer (Bruker Daltonics, Bremen, Germany). Atmospheric-pressure chemical ionization (APCI) mass spectra were recorded in the mass range *m/z* 50–1000 using both positive- and negative-ion modes and the following setting of tuning parameters: target mass *m/z* 250 and compound stability 20% for the alkyl series C_2–C_4, *m/z* 350 and compound stability 100% for the alkyl series C_5–C_{10}, pressure of the nebulizing gas 103.4 kPa, the drying gas flow rate 4 l/min, ion source temperature 350 °C and drying gas temperature 300 °C. The samples were dissolved in acetonitrile and delivered into the system with infusion pump at a flow rate of 5 μl/min.

The analytical HPLC was performed with a Thermo separation products PC 1000, equipped with a reverse-phase column (250 × 4 mm) Nucleosil C18. The elemental analyses were performed by the Central Analytical Service of Department of Organic Chemistry, University of Pardubice.

1,4-Dihydro-1,2,4,5-tetrazine-3,6-dicarboxylic acid (**3**) and dimethyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**4a**) were prepared by known methods[12].

Synthesis of Compounds **5a**–**5c**. General Procedure

An amount of 1 g (5.0 mmol) of **4a**, 60 ml of corresponding alcohol and a catalytic amount of aluminium triethoxide were placed into an autoclave. The autoclave was heated to 180 °C until maximum conversion was reached (HPLC, MeCN–H_2O 4:1). During 1 h alcohol vapours were gradually released (45 ml of the condensate was collected). The autoclave was then cooled to room temperature, the crude product was filtered off and purified by recrystallization from hexane.

Diethyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5a**; *R* = C_2H_5). Yield 46%. M.p. 104–108 °C (ref.[1] 101–102 °C). ^{1}H NMR: 7.51 (NH); 4.39 (OCH_2); 1.39 (CH_3). Positive-ion APCI-MS: *m/z* 229 $[M + H]^+$ (100%), 228 $[M]^+$, 201 $[M + H - C_2H_4]^{+\bullet}$. Positive-ion APCI-MS/MS of *m/z* 229: *m/z* 201 $[M + H - C_2H_4]^+$ (100%), 173 $[M + H - C_4H_8]^+$. Negative-ion APCI-MS: *m/z* 226 $[M - 2]^{-\bullet}$ (100%). For $C_8H_{12}N_4O_4$ (228.2) calculated: 42.10% C, 5.30% H, 24.55% N; found: 41.71% C, 5.38% H, 25.14% N.

Dipropyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5b**; *R* = *n*-C_3H_7). Yield 39%. M.p. 78–81 °C. ^{1}H NMR: 7.53 (NH); 4.28 (OCH_2); 1.76 (CH_2); 0.99 (CH_3). Positive-ion APCI-MS: *m/z* 257 $[M + H]^+$ (100%), 256 $[M]^{+\bullet}$, 215 $[M + H - C_3H_6]^+$. Positive-ion APCI-MS/MS of *m/z* 257: *m/z* 215 $[M + H - C_3H_6]^+$ (100%), 173 $[M + H - C_6H_{12}]^+$. Negative-ion APCI-MS: *m/z* 254 $[M - 2]^{-\bullet}$ (100%). For $C_{10}H_{16}N_4O_4$ (256.3) calculated: 46.87% C, 6.29% H, 21.86% N; found: 46.72% C, 6.27% H, 22.02% N.

Dibutyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5c**; *R* = *n*-C_4H_9). Yield 42%. M.p. 56–61 °C. ^{1}H NMR: 7.49 (NH); 4.31 (OCH_2); 1.73, 1.43 ($(CH_2)_2$); 0.95 (CH_3). Positive-ion APCI-MS: *m/z* 285 $[M + H]^+$ (100%), 284 $[M]^{+\bullet}$. Positive-ion APCI-MS/MS of *m/z* 285: *m/z* 229 $[M + H - C_4H_8]^+$ (100%), 173 $[M + H - C_8H_{16}]^+$. Negative-ion APCI-MS: *m/z* 282 $[M - 2]^{-\bullet}$

(100%). For $C_{12}H_{20}N_4O_4$ (284.3) calculated: 50.69% C, 7.09% H, 19.71% N; found: 50.76% C, 7.04% H, 20.11% N.

Synthesis of Compounds **5d**–**5i**. General Procedure

An amount of 5 g (24.98 mmol) of **4a** was heated with 100 ml of corresponding alcohol and catalytic amount of aluminum triethoxide. A part of the alcohol formed was distilled off from the reaction mixture during 1 h. The rusty brown reaction mixture was then allowed to cool in refrigerator. The obtained crystals were filtered off, recrystallized from hexane and dried in vacuum at 55 °C.

Dipentyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5d**; R = *n*-C_5H_{11}). Yield 71%. M.p. 50–52 °C. ^{1}H NMR: 7.63 (NH); 4.30 (OCH_2); 1.14–1.76 ((CH_2)$_3$); 0.89 (CH_3). ^{13}C NMR: 158.6 (COO); 138.2 (N=C-N); 67.1 (OCH_2); 28.1, 25.2, 22.3 ((CH_2)$_3$); 13.8 (CH_3). Positive-ion APCI-MS: *m/z* 313 $[M + H]^+$ (100%), 312 $[M]^{+\bullet}$, 243 $[M + H - C_5H_{10}]^+$, 173 $[M + H - C_{10}H_{20}]^+$. Positive-ion APCI-MS/MS of *m/z* 313: *m/z* 243 $[M + H - C_5H_{10}]^+$ (100%), 173 $[M + H - C_{10}H_{20}]^+$. Negative-ion APCI-MS: *m/z* 310 $[M - 2]^-$ (100%). For $C_{14}H_{24}N_4O_4$ (312.4) calculated: 53.83% C, 7.74% H, 17.94% N; found: 54.15% C, 7.58% H, 17.73% N.

Dihexyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5e**; R = *n*-C_6H_{13}). Yield 70%. M.p. 56–60 °C. ^{1}H NMR: 7.61 (NH); 4.30 (OCH_2); 1.34–1.77 ((CH_2)$_4$); 0.91 (CH_3). ^{13}C NMR: 158.6 (COO); 138.3 (N=C-N); 67.2 (OCH_2); 27.9, 27.7, 22.1 ((CH_2)$_4$); 13.7 (CH_3). Positive-ion APCI-MS: *m/z* 341 $[M + H]^+$ (100%), 340 $[M]^{+\bullet}$, 257 $[M + H - C_6H_{12}]^+$. Positive-ion APCI-MS/MS of *m/z* 341: *m/z* 257 $[M + H - C_6H_{12}]^+$ (100%), 173 $[M + H - C_{12}H_{24}]^+$. Negative-ion APCI-MS: *m/z* 338 $[M - 2]^{-\bullet}$ (100%). For $C_{16}H_{28}N_4O_4$ (340.4) calculated: 56.45% C, 8.29% H, 16.46% N; found: 56.95% C, 8.22% H, 16.20% N.

Diheptyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5f**; R = *n*-C_7H_{15}). Yield 66%. M.p. 58–61 °C. ^{1}H NMR: 7.60 (NH); 4.30 (OCH_2); 1.24–1.74 ((CH_2)$_5$); 0.88 (CH_3). ^{13}C NMR: 158.6 (COO); 138.3 (N=C-N); 67.3 (OCH_2); 31.5, 29.0, 28.9, 28.3, 25.6, 22.5 ((CH_2)$_5$); 14.0 (CH_3). Positive-ion APCI-MS: *m/z* 369 $[M + H]^+$ (100%), 368 $[M]^{+\bullet}$, 271 $[M + H - C_7H_{14}]^+$. Positive-ion APCI-MS/MS of *m/z* 369: *m/z* 271 $[M + H - C_7H_{14}]^+$ (100%), 173 $[M + H - C_{14}H_{28}]^+$. Negative-ion APCI-MS: *m/z* 366 $[M - 2]^{-\bullet}$ (100%). For $C_{18}H_{32}N_4O_4$ (368.5) calculated: 58.67% C, 8.75% H, 15.20% N; found: 58.94% C, 8.81% H, 15.27% N.

Dioctyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5g**; R = *n*-C_8H_{17}). Yield 70%. M.p. 67–69 °C. ^{1}H NMR: 7.57 (NH-, $^1J(^{15}H,^1H)$ = 87.9); 4.30 (OCH_2); 1.24–1.74 ((CH_2)$_6$); 0.88 (CH_3). ^{13}C NMR: 158.6 (COO); 138.3 (N=C-N); 67.3 (OCH_2); 31.5, 29.0, 28.9, 28.3, 25.6, 22.5 ((CH_2)$_6$); 14.0 (CH_3). ^{15}N NMR: –251.4 (-NH-); –102.8 (-N=). Positive-ion APCI-MS: *m/z* 397 $[M + H]^+$ (100%), 396 $[M]^{+\bullet}$, 285 $[M + H - C_8H_{16}]^+$. Positive-ion APCI-MS/MS of *m/z* 397: *m/z* 285 $[M + H - C_8H_{16}]^+$ (100%), 173 $[M + H - C_{16}H_{32}]^+$. Negative-ion APCI-MS: *m/z* 394 $[M - 2]^{-\bullet}$ (100%). For $C_{20}H_{36}N_4O_4$ (396.5) calculated: 60.58% C, 9.15% H, 14.13% N; found: 60.98% C, 8.99% H, 13.92% N.

Dinonyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5h**; R = *n*-C_9H_{19}). Yield 85%. M.p. 67–70 °C. ^{1}H NMR: 7.59 (NH); 4.30 (OCH_2); 1.26–1.76 ((CH_2)$_7$); 0.88 (CH_3). ^{13}C NMR: 158.6 (COO); 138.2 (N=C-N); 67.1 (OCH_2); 31.7, 29.3, 29.1, 29.0, 28.3, 25.6, 22.5 ((CH_2)$_7$); 14.0 (CH_3). Positive-ion APCI-MS: *m/z* 425 $[M + H]^+$ (100%), 424 $[M]^{+\bullet}$, 299 $[M + H - C_9H_{18}]^+$. Positive-ion APCI-MS/MS of *m/z* 425: *m/z* 299 $[M + H - C_9H_{18}]^+$ (100%), 173 $[M + H - C_{18}H_{36}]^+$. Negative-ion APCI-MS: *m/z* 422 $[M - 2]^{-\bullet}$ (100%). For $C_{22}H_{40}N_4O_4$ (424.6) calculated: 62.24% C, 9.50% H, 13.20% N; found: 62.58% C, 9.46% H, 13.22% N.

Didecyl 1,4-dihydro-1,2,4,5-tetrazine-3,6-dicarboxylate (**5i**; *R* = *n*-$C_{10}H_{21}$). Yield 79%. M.p. 76–77 °C. ^{1}H NMR: 7.54 (NH); 4.30 (OCH_2); 1.26–1.76 ((CH_2)$_8$); 0.88 (CH_3). ^{13}C NMR: 158.6 (COO); 138.3 (N=C-N); 67.2 (OCH_2); 31.8, 29.4, 29.3, 29.2, 29.1, 28.3, 25.6, 22.6 ((CH_2)$_8$); 14.0 (CH_3). Positive-ion APCI-MS: *m/z* 453 $[M + H]^+$ (100%), 452 $[M]^{+\bullet}$, 313 $[M + H - C_{10}H_{20}]^+$. Positive-ion APCI-MS/MS of *m/z* 453: *m/z* 313 $[M + H - C_{10}H_{20}]^+$ (100%), 173 $[M + H - C_{20}H_{40}]^+$. Negative-ion APCI-MS: *m/z* 450 $[M - 2]^{-\bullet}$ (100%). For $C_{24}H_{44}N_4O_4$ (452.6) calculated: 63.69% C, 9.80% H, 12.38% N; found: 63.52% C, 9.98% H, 12.41% N.

Synthesis of Compounds **6a**–**6i**. General Procedure

A solution of 5 g of **5a**–**5i** in 100 ml of dry dichloromethane was cooled to 0 °C. A stream of nitrous gases was bubbled into the reaction mixture with stirring for 30 min. The reaction mixture was stirred for another 1 h. The clear yellow solution gradually turned to bright red. A large part of the solvent was removed, and the red precipitate was filtered off.

Diethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6a**; *R* = C_2H_5). Yield 96%. M.p. 103–106 °C (ref.[21] 105–106 °C). ^{1}H NMR: 4.69 (OCH_2); 1.54 (CH_3). Positive-ion APCI-MS: *m/z* 227 $[M + H]^+$ (100%), 226 $[M]^{+\bullet}$, 199 $[M + H - C_2H_4]^+$. Positive-ion APCI-MS/MS of *m/z* 227: *m/z* 199 $[M + H - C_2H_4]^+$ (100%). Negative-ion APCI-MS: *m/z* 226 $[M]^{-\bullet}$ (100%).

Dipropyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6b**; *R* = *n*-C_3H_7). Yield 94%. M.p. 72–76 °C. ^{1}H NMR: 4.56 (OCH_2); 1.95 (CH_2); 1.09 (CH_3). Positive-ion APCI-MS: *m/z* 255 $[M + H]^+$ (100%), 254 $[M]^{+\bullet}$. Positive-ion APCI-MS/MS of *m/z* 255: *m/z* 213 $[M + H - C_3H_6]^+$ (100%). Negative-ion APCI-MS: *m/z* 254 $[M]^{-\bullet}$ (100%). For $C_{10}H_{14}N_4O_4$ (254.3) calculated: 47.24% C, 5.55% H, 22.04% N; found: 47.83% C, 5.45% H, 21.94% N.

Dibutyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6c**; *R* = *n*-C_4H_9). Yield 95%. M.p. 50–53 °C. ^{1}H NMR: 4.60 (OCH_2); 1.90, 1.45 ((CH_2)$_2$); 0.95 (CH_3). Positive-ion APCI-MS: *m/z* 283 $[M + H]^+$, 282 $[M]^{+\bullet}$ (100%), 227 $[M + H - C_4H_8]^+$, 171 $[M + H - C_8H_{16}]^+$. Positive-ion APCI-MS/MS of *m/z* 282: *m/z* 226 $[M - C_4H_8]^{+\bullet}$ (100%), 172 $[M - C_8H_{14}]^{+\bullet}$. Negative-ion APCI-MS: *m/z* 282 $[M]^{-\bullet}$ (100%). For $C_{12}H_{18}N_4O_4$ (282.3) calculated: 51.06% C, 6.43% H, 19.85% N; found: 50.88% C, 6.56% H, 19.73% N.

Dipentyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6d**; *R* = *n*-C_5H_{11}). Yield 87%. M.p. 42–45 °C. ^{1}H NMR: 4.62 (OCH_2); 1.35–1.92 ((CH_2)$_3$); 0.93 (CH_3). ^{13}C NMR: 160.2 (COO); 159.3 (N=C-N); 68.4 (OCH_2); 28.1, 27.9, 22.3 ((CH_2)$_3$); 13.9 (CH_3). Positive-ion APCI-MS: *m/z* 311 $[M + H]^+$, 310 $[M]^{+\bullet}$ (100%). Positive-ion APCI-MS/MS of *m/z* 310: *m/z* 240 $[M - C_5H_{10}]^{+\bullet}$ (100%), 172 $[M - C_{10}H_{18}]^{+\bullet}$. Negative-ion APCI-MS: *m/z* 310 $[M]^{-\bullet}$ (100%). For $C_{14}H_{22}N_4O_4$ (310.4) calculated: 54.18% C, 7.15% H, 18.05% N; found: 55.17% C, 7.42% H, 16.83% N.

Dihexyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6e**; *R* = *n*-C_6H_{13}). Yield 94%. M.p. 44–47 °C. ^{1}H NMR: 4.61 (OCH_2); 1.31–1.94 ((CH_2)$_4$); 0.91 (CH_3). ^{13}C NMR: 160.2 (COO); 159.1 (N=C-N); 68.2 (OCH_2); 31.3, 28.3, 25.4, 22.1 ((CH_2)$_4$); 14.0 (CH_3). Positive-ion APCI-MS: *m/z* 339 $[M + H]^+$, 338 $[M]^{+\bullet}$ (100%), 256 $[M - C_6H_{10}]^{+\bullet}$. Positive-ion APCI-MS/MS of *m/z* 338: *m/z* 256 $[M - C_6H_{10}]^{+\bullet}$, 254 $[M - C_6H_{12}]^{+\bullet}$, 172 $[M - C_{12}H_{22}]^{+\bullet}$ (100%). Negative-ion APCI-MS: *m/z* 338 $[M]^{-\bullet}$ (100%). For $C_{16}H_{26}N_4O_4$ (338.4) calculated: 56.79% C, 7.74% H, 16.56% N; found: 57.05% C, 7.79% H, 16.19% N.

Diheptyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6f**; *R* = *n*-C_7H_{15}). Yield 92%. M.p. 52–55 °C. ^{1}H NMR: 4.61 (OCH_2); 1.30–1.92 ((CH_2)$_5$); 0.89 (CH_3). ^{13}C NMR: 160.2 (COO); 159.3 (N=C-N); 67.2 (OCH_2); 31.7, 28.6, 28.4, 25.7, 22.6 ((CH_2)$_5$); 14.1 (CH_3). Positive-ion APCI-MS: *m/z* 367 $[M + H]^+$, 366 $[M]^{+\bullet}$ (100%), 270 $[M - C_7H_{12}]^{+\bullet}$, 172 $[M - C_{14}H_{26}]^{+\bullet}$. Positive-ion APCI-MS/MS of *m/z* 366: *m/z* 270 $[M - C_7H_{12}]^{+\bullet}$ (100%), 172 $[M - C_{14}H_{26}]^{+\bullet}$.

Negative-ion APCI-MS: *m/z* 366 $[M]^{-\bullet}$ (100%). For $C_{18}H_{30}N_4O_4$ (366.5) calculated: 59.00% C, 8.25% H, 15.29% N; found: 59.14% C, 8.17% H, 15.08% N.

Dioctyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6g**; *R* = *n*-C_8H_{17}). Yield 98%. M.p. 63–67 °C. ^{1}H NMR: 4.56 (OCH_2); 1.26–1.89 ($(CH_2)_6$); 0.86 (CH_3). ^{13}C NMR: 160.0 (COO); 159.2 (N=C-N); 68.3 (OCH_2); 31.6, 29.0, 28.3, 28.2, 25.7, 22.6 ($(CH_2)_6$); 14.0 (CH_3). ^{15}N NMR: 14.1 (-N=). Positive-ion APCI-MS: *m/z* 395 $[M + H]^+$, 394 $[M]^{+\bullet}$ (100%), 284 $[M - C_8H_{14}]^{+\bullet}$. Positive-ion APCI-MS/MS of *m/z* 394: *m/z* 284 $[M - C_8H_{14}]^{+\bullet}$ (100%), 172 $[M - C_{16}H_{30}]^{+\bullet}$. Negative-ion APCI-MS: *m/z* 394 $[M]^{-\bullet}$ (100%). For $C_{20}H_{34}N_4O_4$ (394.5) calculated: 60.89% C, 8.69% H, 14.20% N; found: 61.11% C, 8.59% H, 14.32% N.

Dinonyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6h**; *R* = *n*-C_9H_{19}). Yield 97%. M.p. 67–69 °C. ^{1}H NMR: 4.61 (OCH_2); 1.25–1.92 ($(CH_2)_7$); 0.87 (CH_3). ^{13}C NMR: 160.1 (COO); 159.3 (N=C-N); 68.4 (OCH_2); 31.8, 29.4, 29.2, 29.1, 28.4, 25.7, 22.6 ($(CH_2)_7$); 14.0 (CH_3). Positive-ion APCI-MS: *m/z* 423 $[M + H]^+$, 422 $[M]^{+\bullet}$ (100%). Positive-ion APCI-MS/MS of *m/z* 422: *m/z* 298 $[M - C_9H_{16}]^{+\bullet}$ (100%), 172 $[M - C_{18}H_{34}]^{+\bullet}$. Negative-ion APCI-MS: *m/z* 422 $[M]^{-\bullet}$ (100%). For $C_{22}H_{38}N_4O_4$ (422.6) calculated: 62.53% C, 9.06% H, 13.26% N; found: 62.70% C, 9.04% H, 12.95% N.

Didecyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**6i**; *R* = *n*-$C_{10}H_{21}$). Yield 89%. M.p. 76–78 °C. ^{1}H NMR: 4.60 (OCH_2); 1.24–1.89 ($(CH_2)_8$); 0.86 (CH_3). ^{13}C NMR: 160.2 (COO); 159.3 (N=C-N); 68.4 (OCH_2); 31.9, 29.5, 29.4, 29.3, 29.2, 28.4, 25.8, 22.7 ($(CH_2)_8$); 14.0 (CH_3). Positive-ion APCI-MS: *m/z* 451 $[M + H]^+$ (100%), 450 $[M]^{+\bullet}$. Positive-ion APCI-MS/MS of *m/z* 451: *m/z* 313 $[M + H - C_{10}H_{18}]^+$ (100%), 172 $[M - C_{20}H_{38}]^{+\bullet}$. Negative-ion APCI-MS: *m/z* 450 $[M]^{-\bullet}$ (100%). For $C_{24}H_{42}N_4O_4$ (450.6) calculated: 63.97% C, 9.39% H, 12.43% N; found: 64.17% C, 9.48% H, 12.35% N.

The authors are grateful for financial support of this research by the VCI Brasil. M. Lísa and M. Holčapek acknowledge the support of Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM0021627502).

REFERENCES

1. Wei-Xiao Hu, Guo-Wu Rao, Ya-Quan Sun: *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1177.
2. Demirbas A.: *Turk. J. Chem.* **2004**, *18*, 311.
3. Makriyannis A., Deng H.: WO 58636 A2 (2002); *Chem. Abstr.* **2002**, *137*, 140538.
4. Gündisch D., Kämpchen T., Schwarz S., Seitz G., Siegl J., Wegge T.: *Bioorg. Med. Chem.* **2002**, *10*, 1.
5. Mohan Jag: *Indian J. Chem., Sect. B* **2003**, *34*, 1013.
6. Pilgram K. H., Wittsell L. E., Skiles R. D., James A. L., Dawson J. W.: *J. Agric. Food Chem.* **1977**, *25*, 888.
7. Chavez D. E., Hiskey M. A., Naud D. L.: *Propellants, Explosives, Pyrotech.* **2004**, *29*, 209.
8. Coburn M. D., Ott D. G. (United States Department of Energy): U.S. 5281706 (1994); *Chem. Abstr.* **1994**, *120*, 191739.
9. Naud S.: *Synlett* **2004**, 2836.
10. Girardot M., Nomak R., Snyder J. K.: *J. Org. Chem.* **1998**, *63*, 10063.
11. Boger D. L., Panek J. S., Patel M.: *Org. Synth.* **1998**, *9*, 335.
12. Boger D. L., Panek J. S.: *J. Am. Chem. Soc.* **1985**, *107*, 5745.
13. Boger D. L., Coleman R. S., Panek J. S.: *J. Org. Chem.* **1985**, *50*, 5377.

14. Soenen D. R., Zimpleman J. M., Boger D. L.: *J. Org. Chem.* **2003**, *68*, 3593.
15. Sauer J., Heldmann D. K., Hetzenegger J., Krauthan J., Sichert H., Schuster J.: *Eur. J. Org. Chem.* **1998**, 2885.
16. Holland V. R. (Commonwealth Scientific and Industrial Research Organisation): U.S. 5334623 (1994); *Chem. Abstr.* **1994**, *115*, 155287.
17. Zhou X., Kovalev E. G., Klug J. T., Khodorkovsky V.: *Org. Lett.* **2001**, *3*, 1725.
18. Mueller E., Herrdegen L.: *J. Prakt. Chem.* **1921**, *102*, 113.
19. Holčapek M., Lísa M., Volná K., Almonasy N., Přikryl J.: *J. Mass Spectrom.* **2007**, *42*, 1645.
20. Holčapek M., Volná K., Vaněrková D.: *Dyes Pigm.* **2007**, *75*, 156.
21. Mueller E.: *Chem. Ber.* **1908**, *41*, 3122.