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Synthesis of novel intermediates for cyanine dyes by the quaternization of *N*-heterocycles with acrylamide and *N*-alkyl acrylamides

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Abstract

The preparation of several novel important heterocyclic *N*-(2-carbamoyl-ethyl) quaternary salts by 1,4-addition of an appropriate *N*-heterocyclic base to acrylamide or *N*-alkyl acrylamides under different reaction conditions in the presence of hydrochloric, hydrobromic or perchloric acid is described. The products have an activated group at 2- or 4-position and are typical precursors of different types of cyanine dyes. The products were characterized by ^{1}H NMR, IR spectra, HPLC–MS spectrometry and elemental analysis.

Keywords: Acrylamide; *N*-Alkyl acrylamides; Quaternary salts; Cyanine dyes; Nucleic acids; Fluorescence

1. Introduction

In recent years, there has been a growing interest in the synthesis and application of mono- and polymethine dyes as noncovalent labels for nucleic acid detection [1–3]. These dyes have considerable scientific, practical and commercial significance in nucleic acid research as well as in clinical and environmental analysis [4–13]. Our research in this area [14–21] has prompted us to search for new intermediates and dyes. Several heterocyclic *N*-alkyl quaternary salts have been described in the literature [22–25] as isolated or non-isolated cyanine precursors, where the alkyl group is mainly methyl, ethyl, pentyl, hexyl or decyl. In another work [26] 5,6-dimethoxy-2-methylbenzothiazole and an equimolar amount of 3-iodopropionamide were heated at 100 °C for 1 h and the resulting 3-(2-carbamoylethyl)-5,6-dimethoxy-2-methylbenzothiazolium iodide was directly used as dye intermediate.

The known methods for quaternization with acrylamides are performed with nitrogen-containing heterocyclic azo-dyes [27–30]. To the best of our knowledge, the preparation of precursors of cyanine dyes by means of 1,4-addition of *N*-heterocyclic compounds to acrylamides has not been investigated so far.

2. Experimental

Melting points were determined on a Kofler apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Bruker 250 MHz instrument in DMSO-d_6. IR spectra were recorded with a FTIR Bruker 22. HPLC–MS spectra were obtained on an Angilent 1100 apparatus with a nucleosil RP 18 column and with

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solvent – 80% water, 19.95% acetonitrile and 0.05% trifluoroacetic acid. Acrylamide **1a** was a commercial product. Diacetone acrylamide **1b** and *N*-cyclohexylacrylamide **1c** were prepared from acrylonitrile and the appropriate alcohol in concentrated sulfuric acid [30]. Lepidine, 4-chloroquinoline, 4,7-dichloroquinoline, 2-methylbenzothiazole, 2-methylbenzoxazole, γ-picoline and 4-chloropyridinium chloride **2d** were commercial products.

*2.1. Preparation of heterocyclic salts **2a–2i** (except **2d**)*

0.1 mol 70% $HClO_4$ (or 48% HBr) was dropped into a diethyl ether solution of an equimolar amount of the respective heterocyclic base. The resulting precipitate of the bromide or perchlorate salt was suction filtered and dried in vacuum. The yields were quantitative.

*2.2. Preparation of compounds **3a** and **3b***

2.2.1. Procedure A

2-Methylbenzothiazole (8 mmol), 20 ml acetic acid, 12 mmol 48% HBr or 72% $HClO_4$ and 20 mmol acrylamide **1a** were mixed in a 50 ml round-bottomed flask fitted with reflux condenser and mechanical stirrer and the mixture was refluxed for 2 h. After cooling to room temperature, the reaction mixture was poured into 60 ml diethyl ether and left for 48–72 h at 0 °C. The crystalline products were suction filtered and air dried. Compounds **3a** and **3b** were recrystallized from methanol.

3-(2-Carbamoylethyl)-2-methylbenzothiazolium bromide **3a**. Yield: 53%. M.p. 209–210 °C. ^1H NMR (DMSO) δ: 2.82 (t, $^3J_{HH} = 6.8$ Hz, 2H, CH_2), 3.26 (s, 3H, CH_3), 4.92 (t, $^3J_{HH} = 6.8$ Hz, 2H, N^+CH_2), 7.12 (s, 1H, NH_2), 7.54 (s, 1H, NH_2), 7.83–8.54 (m, 4H, Ar).

3-(2-Carbamoylethyl)-2-methylbenzothiazolium perchlorate **3b**. Yield: 59%. M.p. 166–167 °C. ^1H NMR (DMSO) δ: 2.81 (t, $^3J_{HH} = 6.8$ Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 4.93 (t, $^3J_{HH} = 6.8$ Hz, 2H, N^+CH_2), 7.10 (s, 1H, NH_2), 7.53 (s, 1H, NH_2), 7.80–8.45 (m, 4H, Ar).

*2.3. Preparation of compounds **3a**, **3b** and **4a***

2.3.1. Procedure B

A mixture of 8.00 mmol 2-methylbenzothiazolium hydrogen bromide **2a** or 2-methylbenzothiazolium hydrogen perchlorate **2b**, or 4-chloropyridinium hydrogen chloride **2d**, 20 mmol acrylamide **1a**, 20 ml acetic acid and one drop of 2-methylbenzothiazole (or pyridine in the case of **2d**) were refluxed for 2 h in a two-neck round-bottomed flask equipped with a mechanical stirrer, reflux condenser and thermometer. After cooling to room temperature, the acetic acid solution was poured into 60 ml diethyl ether and left for 48 h at 0 °C. The white crystalline product was suction filtered and air dried. All reaction products were recrystallized from methanol except for **4a**, where methanol:diethyl ether (1:1) was used.

3-(2-Carbamoylethyl)-2-methylbenzothiazolium bromide **3a**. Yield: 80%. M.p. 211–212 °C. ^1H NMR (DMSO) δ: 2.81 (t, $^3J_{HH} = 6.8$ Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 4.92 (t, $^3J_{HH} = 6.8$ Hz, 2H, N^+CH_2), 7.09 (s, 1H, NH_2), 7.54 (s, 1H, NH_2), 7.80–8.50 (m, 4H, Ar). IR (KBr) cm^{-1}: 1672 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 221, M^+ (found) = 221.

3-(2-Carbamoylethyl)-2-methylbenzothiazolium perchlorate **3b**. Yield: 83%. M.p. 168–169 °C. ^1H NMR (DMSO) δ: 2.80 (t, $^3J_{HH} = 6.8$ Hz, 2H, CH_2), 3.24 (s, 3H, CH_3), 4.91 (t, $^3J_{HH} = 6.8$ Hz, 2H, N^+CH_2), 7.10 (s, 1H, NH_2), 7.51 (s, 1H, NH_2), 7.77–8.44 (m, 4H, Ar). IR (KBr) cm^{-1}: 1656 ($CONH_2$). For $C_{11}H_{13}ClN_2O_5S$ (320.75) Calcd.: C, 41.19; H, 4.09; N, 8.73. Found: C, 41.80; H, 4.30; N, 8.30.

1-(2-Carbamoylethyl)-4-chloropyridinium chloride **4a**. Yield: 91%. M.p. 158–161 °C. ^1H NMR (DMSO) δ: 2.75 (t, $^3J_{HH} = 6.5$ Hz, 2H, CH_2), 4.52 (t, $^3J_{HH} = 6.5$ Hz, 2H, N^+CH_2), 7.03 (s, 1H, NH_2), 7.39 (d, $^3J_{HH} = 5.6$ Hz, 2H, Ar), 7.63 (s, 1H, NH_2), 8.65 (d, $^3J_{HH} = 5.6$ Hz, 2H, Ar). IR (KBr) cm^{-1}: 1678 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 185, M^+ (found) = 185.

*2.4. Preparation of 3-[2-(1,1-dimethyl-3-oxobutylcarbamoyl)-ethyl]-2-methylbenzothiazolium perchlorate **3c***

2.4.1. Procedure A

Two grams (8.69 mmol) of 2-methylbenzothiazolium hydrogen bromide **2a**, 0.77 g (22 mmol) diacetone acrylamide **1b**, 30 ml acetic acid and one drop of 2-methylbenzothiazole were added to a two-neck round-bottomed flask equipped with a magnetic stirrer, reflux condenser and thermometer. The reaction mixture was heated at 90 °C for 2 h. After cooling to room temperature, the acetic acid solution was poured into 60 ml diethyl ether. The resulting oily residue was separated by decantation, dissolved in 20 ml methanol and the solution was poured into 20 ml of a saturated aqueous solution of sodium perchlorate. After 48 h at 0 °C, the white crystalline product was suction filtered and air dried. The reaction product was recrystallized from methanol. Yield: 40%. M.p. 140–141 °C.^1H NMR (DMSO) δ: 1.14 (s, 6H, 2 × CH_3), 1.90 (s, 3H, $COCH_3$), 2.79 (t, 2H, $^3J_{HH} = 6.4$ Hz, CH_2CO), 2.79 (s, 2H, CH_2CO), 3.24 (s, 3H, CH_3), 4.90 (t, 2H, $^3J_{HH} = 6.4$ Hz, N^+CH_2), 7.76–8.44 (m, 5H, NH + Ar). IR (KBr) cm^{-1}: 1691 (CONH). For $C_{16}H_{21}ClN_2O_6S$ (320.75) Calcd.: C, 47.47; H, 5.23; N, 6.92. Found: C, 47.3; H, 5.37; N, 7.04.

2.4.2. Procedure B

One gram (4 mmol) of 2-methylbenzothiazolium hydrogen perchlorate **2b**, 1.69 g (10 mmol) diacetone acrylamide **1b**, 20 ml acetic acid and one drop 2-methylbenzothiazole were added to a reaction vessel and left in the dark at room temperature for 2 months (TLC monitoring). The mixture was poured into 50 ml diethyl ether and the white crystalline product was filtered, washed with ether and air dried. Yield: 84%. M.p. 139–141 °C. ^{1}H NMR (DMSO) δ: 1.15 (s, 6H, $2 \times CH_3$), 1.91 (s, 3H, $COCH_3$), 2.79 (s, 2H, CH_2CO), 2.79 (t, 2H, $^3J_{HH} = 6.4$ Hz, CH_2CO), 3.24 (s, 3H, CH_3), 4.90 (t, 2H, $^3J_{HH} = 6.4$ Hz, N^+CH_2), 7.76–8.44 (m, 5H, NH + Ar). HPLC–MS *m/z* (%): M^+ (calcd) = 319, M^+ (found) = 319.

2.5. Preparation of 3-(2-cyclohexylcarbamoylethyl)-2-methylbenzothiazolium perchlorate *3d*

Two grams (8 mmol) of 2-methylbenzothiazolium hydrogen perchlorate **2b**, 3.06 g (20 mmol) *N*-cyclohexylacrylamide **1c**, 20 ml acetic acid and one drop of 2-methylbenzothiazole were heated at 90 °C for 3 h. The reaction pathway was the same as that for the preparation of **3a**. Yield: 65%. M.p. 150–151 °C. ^{1}H NMR (DMSO) δ: 0.93–1.20 (m, 5H, Alyf), 1.46–1.59 (m, 5H, Alyf), 2.77 (t, 2H, $^3J_{HH} = 6.5$ Hz, CH_2CO), 3.23 (s, 3H, CH_3), 3.23–3.42 (m, 1H, NCH), 4.94 (t, 2H, $^3J_{HH} = 6.5$ Hz, N^+CH_2), 7.79–8.44 (m, 5H, NH + Ar). IR (KBr) cm^{-1}: 1670 (CONH). HPLC–MS *m/z* (%): M^+ (calcd) = 303, M^+ (found) = 303.

2.6. Preparation of 3-(2-carbamoylethyl)-2-methylbenzoxazolium bromide *3e*

Two grams (1.8 ml, 15 mmol) of 2-methylbenzoxazole, 15 ml acetic anhydride, 5 ml acetic acid, 2.2 ml (19.45 mmol) 48% HBr and 2.67 g (38 mmol) acrylamide **1a** were mixed in a reaction vessel equipped with thermometer, reflux condenser and electromagnetic stirrer, and heated in an oil bath at 90 °C for 3 h. The reaction mixture was then treated in a similar way as described for the preparation of **3a**. The product **3e** was recrystallized from methanol:diethyl ether (1:1). Yield: 90%. M.p. 197–201 °C. ^{1}H NMR (DMSO) δ: 3.12 (s, 3H, CH_3), 3.24 (t, 2H, $^3J_{HH} = 6.6$ Hz, N^+CH_2), 4.77 (t, 2H, $^3J_{HH} = 6.6$ Hz, N^+CH_2), 6.98–8.23 (m, 6H, NH_2 + Ar). IR (KBr) cm^{-1}: 1693 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 205, M^+ (found) = 205 (30%), 247 (5%), 223, 265.

2.7. Preparation of products *4b* and *5a–5d*

39 mmol of the respective *N*-heterocyclic salt (lepidinium hydrogen bromide **2f** or perchlorate **2g**, γ-picolinium hydrogen bromide **2e**, 4-chloroquinolinium **2h** or 4,7-dichloroquinolinium **2i** hydrogen bromide), 70 ml acetic acid, 98 mmol acrylamide **1a** and two drops (or a few crystals) of the respective free *N*-heterocyclic base were mixed in a 100 ml round-bottomed flask fitted with reflux condenser and mechanical stirrer and the mixture was refluxed for 3 h. After cooling to room temperature, the reaction mixture was poured into 150 ml diethyl ether and left for 48–72 h at 0 °C. The crystalline products were suction filtered and air dried. All compounds were recrystallized from methanol.

1-(2-Carbamoylethyl)-4-methylpyridinium bromide **4b**. Yield: 90%. M.p. 211–213 °C. ^{1}H NMR (DMSO) δ: 2.58 (s, 3H, CH_3), 2.87 (t, 2H, $^3J_{HH} = 6.4$ Hz, CH_2CO), 4.74 (t, 2H, $^3J_{HH} = 6.4$ Hz, N^+CH_2), 7.03 (s, 1H, NH), 7.60 (s, 1H, NH), 7.97 (d, 2H, $^3J_{HH} = 6.3$ Hz, Ar), 8.97 (d, 2H, $^3J_{HH} = 6.5$ Hz, Ar). IR (KBr) cm^{-1}: 1671 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 165, M^+ (found) = 165.

1-(2-Carbamoylethyl)-4-methylquinolinium bromide **5a**. Yield: 82%. M.p. 203–204 °C. ^{1}H NMR (DMSO) δ: 2.88 (t, 2H, $^3J_{HH} = 6.6$ Hz, CH_2CO), 3.00 (s, 3H, CH_3), 5.22 (t, 2H, $^3J_{HH} = 6.6$ Hz, N^+CH_2), 7.06 (s, 1H, NH), 7.53 (s, 1H, NH), 8.01–9.40 (m, 6H, Ar). IR (KBr) cm^{-1}: 1678 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 215, M^+ (found) = 215.

1-(2-Carbamoylethyl)-4-methylquinolinium perchlorate **5b**. Yield: 85%. M.p. 145–147 °C. ^{1}H NMR (DMSO) δ: 2.98 (s, 3H, CH_3), 3.03 (t, 2H, $^3J_{HH} = 6.9$ Hz, CH_2CO), 5.18 (t, 2H, $^3J_{HH} = 6.9$ Hz, N^+CH_2), 7.04 (s, 1H, NH), 7.45 (s, 1H, NH), 8.26–9.39 (m, 6H, Ar). IR (KBr) cm^{-1}: 1685 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 215, M^+ (found) = 215.

1-(2-Carbamoylethyl)-4-chloroquinolinium bromide **5c**. Yield: 79%. M.p. 124–127 °C. ^{1}H NMR (DMSO) δ: 2.75 (t, 2H, $^3J_{HH} = 6.5$ Hz, CH_2CO), 4.92 (t, 2H, $^3J_{HH} = 6.5$ Hz, N^+CH_2), 7.04 (s, 1H, NH), 7.45 (s, 1H, NH), 7.82–8.42 (m, 6H, Ar). IR (KBr) cm^{-1}: 1667 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 235, M^+ (found) = 235 (95%), M^+ = 164 (5%).

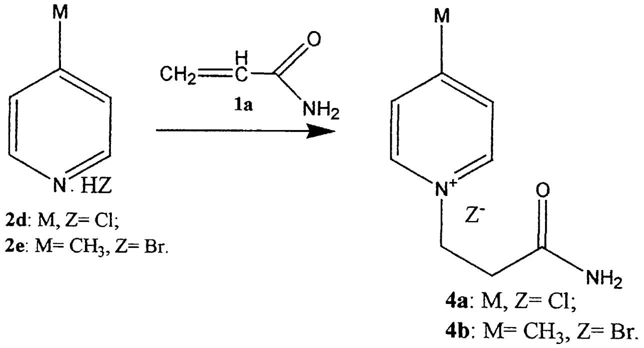
1-(2-Carbamoylethyl)-4,7-dichloroquinolinium bromide **5d**. Yield: 94%. ^{1}H NMR (DMSO) δ: 2.90 (t, 2H, $^3J_{HH} = 6.6$ Hz, CH_2CO), 5.24 (t, 2H, $^3J_{HH} = 6.6$ Hz, N^+CH_2), 7.06 (s, 1H, NH), 7.09 (s, 1H, NH), 7.94–8.61 (m, 5H, Ar). IR (KBr) cm^{-1}: 1678 ($CONH_2$). HPLC–MS *m/z* (%): M^+ (calcd) = 269, M^+ (found) = 269 (90%), M^+ = 251 (5%), M^+ = 194 (5%).

3. Results and discussion

In this paper, we present a study of the reaction conditions for the synthesis of several novel important precursors of cyanine dyes. The standard route to the quaternization of *N*-heterocyclic azo-dyes with acrylamides is a reaction in acetic acid in the presence of Brönsted acids (concentrated hydrochloric, hydrobromic, sulfuric or perchloric acid), which introduces a certain amount of water into the reaction mixture [27–29]. In our case this simplifies the procedure to a great extent, but leads to yields in the range of only 50–60%. The synthesis of 3-(2-carbamoylethyl)-2-methylbenzothiazolium bromide **3a**, 3-(2-carbamoylethyl)-2-methylbenzothiazolium perchlorate **3b** and 1-(2-carbamoylethyl)-4-chloropyridinium chloride **4a** was carried out in acetic acid solution, starting from 2-methylbenzothiazolium hydrogen bromide **2a** or perchlorate **2b** and 4-chloropyridinium hydrogen chloride **2d** in an excess of acrylamide **1a** and in the presence of catalytic amounts of the respective base. In this way, we could carry out the reaction in the absence of water. This increased the reaction yields up to 80–90% (Schemes 1 and 2, Table 1).

This observation provoked us to synthesize products **4a** and **5a**–**5d** in a similar way and on a larger scale (Schemes 2 and 3). The quaternization of **2a** with diacetone acrylamide **1b** was performed essentially in the aforementioned way. However, the reaction temperature should not exceed 90 °C and the reaction time should remain below 2 h in order to avoid cleavage of the *N*-alkyl moiety from the amide group and formation of **3a** as sole reaction product.

Our attempts to perform the quaternization of **2b** with **1b** in the same way afforded only the dealkylation product **3b**. Reaction at 60 °C for 4 h yielded only the starting compounds **1b** and **2b** according to the ^{1}H NMR spectrum. Prolonged heating of the reaction mixture resulted in a white gummy residue – probably polyacrylamide. However, keeping the reaction mixture for 2 months at room temperature provided the desired compound **3c** in excellent yield and high purity.



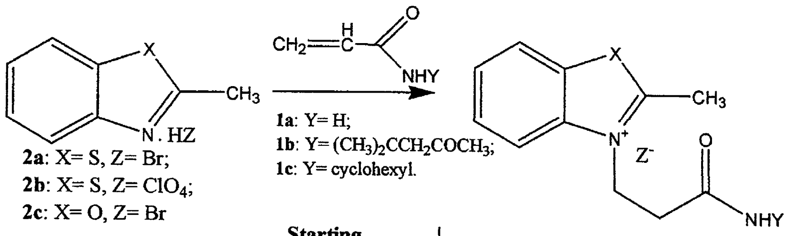
Scheme 2. Synthesis of heterocyclic *N*-carbamoylethyl quaternary salts **4a** and **4b**.

The reaction of 2-methylbenzothiazolium hydrogen perchlorate **2b** with the more stable *N*-cyclohexylacrylamide **1c** under the same (Procedure A, preparation of product **3c**) conditions occurred without dealkylation (Scheme 1, Table 1).

Our synthesis of 3-(2-carbamoylethyl)-2-methylbenzoxazolium bromide **3e**, which is the intermediate for many cyanine dyes (for example YO and the YOYO-family), from 2-methylbenzoxazole and **1a** is important because of the instability of the benzoxazolium quaternary salts in many solvents [18]. The reaction could be performed successfully in acetic anhydride due to the stability of the benzoxazole ring and the quaternization product in this medium.

In all these reactions the acetic acid is not just a solvent. It rather inhibits the polymerization of the acrylamides. For this purpose, addition of a small amount of hydroquinone is also helpful.

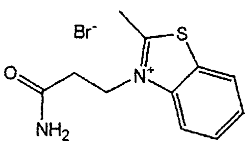
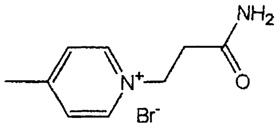
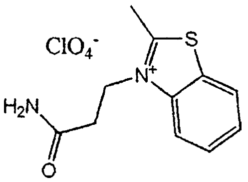
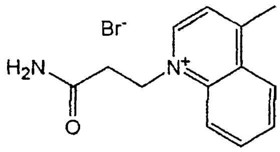
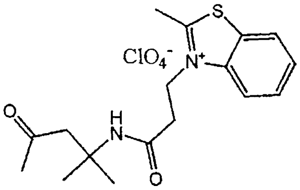
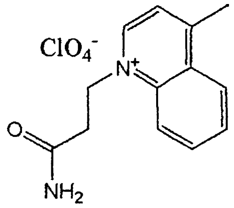
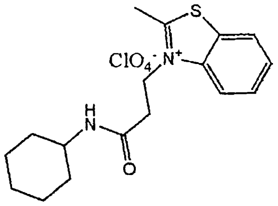
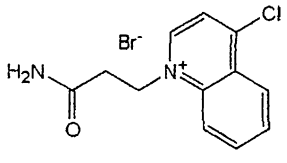
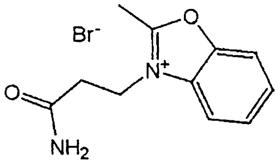
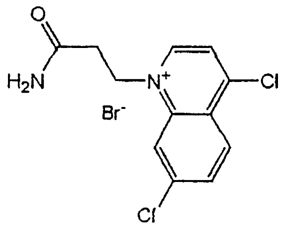
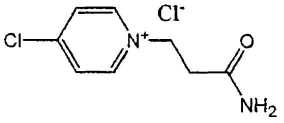
The products were characterized by ^{1}H NMR and IR spectra, HPLC–MS spectrometry and elemental analysis. Compound **5d** is highly hygroscopic which hampers the melting point determination. The products **4a**, **3e**, **5c** and **5d** are unstable on silicagel and at high temperatures in most solvents, which prevents recrystallization and complete purification. Nevertheless, their structures were verified by ^{1}H NMR, IR spectra and HPLC–MS spectrometry. The cyanine dyes derived from these intermediates will be described in another paper. Their

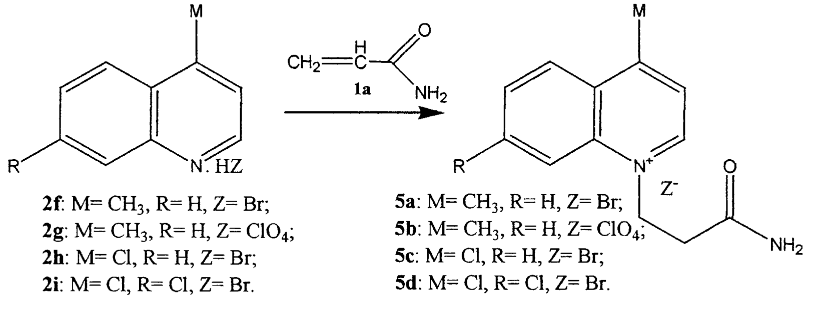


Starting compounds	Product
1a and **2a**	**3a**: X= S, Y= H, Z= Br;
1a and **2b**	**3b**: X= S, Y= H, Z= ClO_4;
1b and **2a** (or **1b** and **2b**)	**3c**: X= S, Y= $(CH_3)_2CCH_2COCH_3$, Z= ClO_4;
1c and **2b**	**3d**: X= S, Y= cyclohexyl, Z= ClO_4;
1a and **2c**	**3e**: X= O, Y= H, Z= Br.

Scheme 1. Synthesis of heterocyclic *N*-carbamoylethyl quaternary salts **3a**–**3e**.

Table 1
Structures of products **3a**–**3k**

Name of a product, No.	Structure	Name of a product, No.	Structure
3-(2-carbamoylethyl)-2-methylbenzothiazolium bromide, **3a**		1-(2-carbamoylethyl)-4-methylpyridinium bromide, **4b**	
3-(2-carbamoylethyl)-2-methylbenzothiazolium perchlorate, **3b**		1-(2-carbamoylethyl)-4-methylquinolinium bromide, **5a**	
3-[2-(1,1-dimethyl-3-oxobutylcarbamoyl)-ethyl]-2-methylbenzothiazolium perchlorate, **3c**		1-(2-carbamoylethyl)-4-methylquinolinium perchlorate, **5b**	
3-(2-cyclohexylcarbamoylethyl)-2-methylbenzothiazolium perchlorate, **3d**		1-(2-carbamoylethyl)-4-chloroquinolinium bromide, **5c**	
3-(2-carbamoylethyl)-2-methylbenzoxazolium bromide, **3e**		1-(2-carbamoylethyl)-4,7-dichloroquinolinium bromide, **5d**	
1-(2-carbamoylethyl)-4-chloropyridinium chloride, **4a**			



2f: M= CH_3, R= H, Z= Br;
2g: M= CH_3, R= H, Z= ClO_4;
2h: M= Cl, R= H, Z= Br;
2i: M= Cl, R= Cl, Z= Br.

5a: M= CH_3, R= H, Z= Br;
5b: M= CH_3, R= H, Z= ClO_4;
5c: M= Cl, R= H, Z= Br;
5d: M= Cl, R= Cl, Z= Br.

Scheme 3. Synthesis of heterocyclic *N*-carbamoylethyl quaternary salts **3g**, **3h**, **3j** and **3k**.

successful preparation lends further support to the intermediate's structures.

4. Conclusions

Eleven novel important heterocyclic *N*-(2-carbamoylethyl) quaternary salts suitable as intermediates for different types of cyanine dyes were prepared by the reaction of 1,4-addition of an appropriate *N*-heterocyclic base to acrylamide or *N*-alkyl acrylamides in the presence of hydrochloric, hydrobromic or perchloric acid and different reaction conditions were investigated.

These improved procedures have the following advantages:

- They are simple (no special apparatus is needed) and reliable;
- The formation of dealkylated products was avoided when diacetone acrylamide **1b** and cyclohexyl acrylamide **1c** were used;
- The formation of polyacrylamides was avoided;
- They can be applied to less basic heterocycles, such as benzothiazole and benzoxazole.

The work on the synthesis of novel monomethine cyanine dyes for nucleic acids detection from the described above precursors is in progress and will be published elsewhere.

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