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The Synthesis of Novel Iodinated Iminodiacetic Acid Analogues as Hepatobiliary Imaging Agents

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Summary. The synthesis and characterization of *N*-[2-[[4-iodo-2,6-bis(1-methylethyl)phenyl]amino]-2-oxoethyl]-*N*-(carboxymethyl)glycine and *N*-[2-[(4-iodo-2,6-diethylphenyl)amino]-2-oxoethyl]-*N*-(carboxymethyl)glycine is presented, as well as a modified and improved synthesis of *N*-[2-[(2, 4-diiodo-6-methylphenyl)amino]-2-oxoethyl]-*N*-(carboxymethyl)glycine. These compounds are new agents for hepatobiliary imaging.

Keywords. *IDA* analogues; Halogenation; NMR spectroscopy; IR spectroscopy; Mass spectroscopy.

Introduction

The halogenated analogues of *N*-(carboxymethyl)glycine (iminodiacetic acid; *IDA*) labeled with technetium-99m have been used recently as diagnostic radiopharmaceutical for hepatobiliary imaging. Numerous derivatives of *IDA* have been synthesized since the discovery of *N*-[2-[(2,6-dimethylphenyl)amino]-2-oxoethyl]-*N*-(carboxymethyl)glycine *HIDA* [1, 2] in order to obtain compounds of better hepatobiliary properties. Biokinetics of these compounds depends strongly on molecular mass, lipophilicity, protein binding, and the substitution of the aromatic system [3–5]. Introduction of halogen in the aromatic system of N-substituted *IDA* derivatives increases the relative molecular mass and lipophilicity leading to increased biliary excretion and reduction of urinary excretion and competition with bilirubin. The hepatobiliary agents 3-iodo-2,6-diethyl-*IDA* (*IODIDA*) and 3-bromo-2,4,6-trimethyl-*IDA* (mebrofenin) are widely used in hepatobiliary imaging [6].

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Unlike chlorine and bromine, iodine is unreactive towards most aromatic substrates; thus, a more powerful iodinating species like iodonium equivalents (I^+) or reagent combinations that generate electrophilic iodine species [7, 8] are required for direct aromatic iodination. The moderate reactivity of iodine with aromatic substrates mandates for utilisation of an oxidizing agent (H_2O_2, HIO_3, HgO, CH_3CO_3H, $HgCl_2$, KI) and a base (NH_4OH, $NaHCO_3$, KOH). It has been also reported that aromatic iodination with molecular iodine was catalyzed by stoichiometric amounts of metal halides, such as $AlCl_3$–$CuCl_2$ [9] or $SbCl_5$ [10]. The direct iodination has been carried out by using NH_4I and catalytic amounts of $NOBF_4$ in CF_3COOH/CH_2Cl_2 or CF_3COOH/CH_3COOH with molecular oxygen [11]. Direct iodination methods have been intensively developed in recent years by using iodonium donating systems, such as iodine-nitrogen dioxyde [12], *N*-iodosuccinimide [13], ICl (alone or in the presence of $ZnCl_2$, pyridine, or benzyltrimethylammonium chloride), or benzyltriethylammonium dichloroiodate/sodium bicarbonate as a mild iodinating reagent for benzenamine [14]. *Kajigaeshi et al.* [15] reported the use of benzyltrimethylammonium dichloroiodate (*BTMA* ICl_2) as an iodinating agent for aromatic amines.

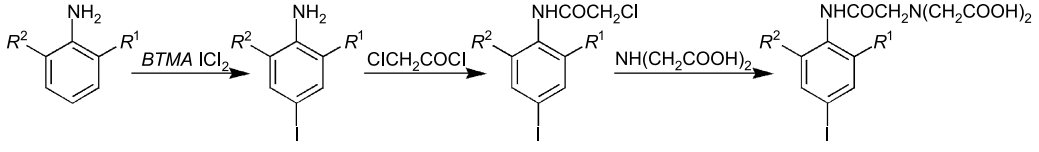
Results and Discussions

The synthesis of the compounds **1**, **2**, and **3** in three steps is outlined in (Scheme 1). The first step consists of a iodination by means of *BTMA* ICl_2 according to Ref. [15]. The reaction of the benzenamines with one or two equivalents of *BTMA* ICl_2 in a dichloromethane-methanol mixture in presence of calcium carbonate gave the 4-iodo- or 2,4-diiodobenzenamines. This method gives good yields, no secondary products are obtained, and the reaction is carried out under neutral conditions at room temperature.

To obtain the target compounds, the iodinated benzenamines **1a**, **2a**, and **3a** were reacted with chloroacetylchloride in acetone. The products **1b**, **2b**, and **3b** were subsequently condensed with *IDA* in refluxing aqueous ethanol to yield **1**, **2**, and **3**.

N-[2-[(2,4-Diiodo-6-methylphenyl)amino]-2-oxoethyl]-*N*-(carboxymethyl)glycine (**3**) has been synthesized recently and labeled with technetium-99m [16]. The iodination reaction was performed with I_2 in aqueous $NaHCO_3$ solution, and the yield of the precursor **3a** was 51%. The synthesis of **3a**, using *BTMA* ICl_2 as iodinating agent as described above resulted in an improvement to 76% yield.

The structures of **1**, **2**, and **3** could be confirmed by their elemental analyses and spectroscopic data (UV, IR, ^{1}H NMR, ^{13}C NMR, and GC-mass spectrometry; see



1a:R^1=R^2=-$CH(CH_3)_2$ **1b**:R^1=R^2=-$CH(CH_3)_2$ **1**:R^1=R^2=-$CH(CH_3)_2$
2a:R^1=R^2=-CH_2CH_3 **2b**:R^1=R^2=-CH_2CH_3 **2**:R^1=R^2=-CH_2CH_3
3a:R^1=-I; R^2=-CH_3 **3b**:R^1=-I; R^2=-CH_3 **3**:R^1=-I; R^2=-CH_3

Scheme 1

Experimental). The purity of the compounds was confirmed by melting point, GC, and TLC. The obtained products **1**, **2**, and **3** are promising for biological evaluation studies.

Experimental

Melting points were determined on a Boetius PHMK 05 apparatus. UV spectra were recorded on a CINTRA 20. IR spectra (KBr): FTS 3000 MX (BIO-RAD, Excalibur). ^{1}H NMR and ^{13}C NMR spectra were recorded on a GEMINI-200 at 200 and 50 MHz using *DMSO*-d_6 as solvent and *TMS* as internal standard, chemical shifts in δ (ppm). Mass spectra: Hewlett Packard GCD series II (70 eV) HP-5MS (cross-linked 5% PH-ME-Siloxane), 30 m × 0.25 mm × 0.25 µm, carrier gas: hydrogen, 0.9 cm^3/min, T injector 250°C, T detector 280°C, T column from 150°C to 280°C (10°C/min); or HP 5090 and 5080 (70 eV), HPL 12 m × 0.2 mm, carrier gas: He, 0.6 cm^3/min, T detector 280°C. Elemental analyses were conducted using the Elemental Analyser Vario EL III CHNS/O, their results were found to be in good agreement (±0.16%) with the calculated values. Thin layer chromatography (TLC): TLC plates (Merck, silica gel 60 F_{254}, 0.25 mm, 200 × 200 mm; the compounds were detected in UV light at 254 nm). All reagents were obtained from commercial sources and used as received.

4-Iodo-2,6-bis(1-methylethyl)benzenamine (**1a**, $C_{12}H_{18}IN$)

To the solution of 1.77 g of 2,6-bis(1-methylethyl)benzenamine (10 mmol) in CH_2Cl_2 (50 cm^3) – CH_3OH (25 cm^3), were added 3.48 g of *BTMA* ICl_2 (10 mmol) and 1.1 g of $CaCO_3$ (11 mmol). The mixture was stirred for 6 h at room temperature. During that period, the colour of the solution gradually changed from yellow to light brown. On completion (monitored by TLC) the excess of $CaCO_3$ was filtered off and the filtrate was concentrated. To the obtained residue, 30 cm^3 of $NaHSO_3$ solution (5%) were added. The mixture was extracted with 4 × 30 cm^3 of diethyl ether, the ether layer was dried (Na_2SO_4), filtered, and evaporated *in vacuo* to give 2.6 g (85%) of a yellowish-brown oily mass. The crude product was used in next synthetic step. UV (methanol, $c = 2 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 208 (34000), 249 (12200), 295 (3200) nm (mol^{-1} dm^3 cm^{-1}); TLC: $R_f = 0.45$ (*n*-hexane:benzene = 3:7), $R_f = 0.85$ ($CHCl_3$); GC: single peak at $R_t = 7.4$ min; MS (70 eV): m/z (%) = 303 (83.7) [M^+], 288 (100.0), 161 (7.4), 146 (25.0), 130 (9.8), 115 (7.1), 91 (6.2).

N-[4-Iodo-2,6-bis(1-methylethyl)phenyl]-2-chloroacetamide (**1b**, $C_{14}H_{19}ICINO$)

To the solution of crude **1a** in 40 cm^3 of acetone 2 cm^3 of chloroacetylchloride (25 mmol) were added. The mixture was cooled at 0–4°C with constant stirring for 2 h. To that solution, 30 cm^3 of HCl (8%) were added and stirred for 30 min. The precipited **1b** was filtered off under vacuum, dried, and recrystallized from ethanol to yield 2.47 g (65%). Mp 213–214°C; TLC: $R_f = 0.14$ (*n*-hexane:benzene = 3:7), $R_f = 0.62$ ($CHCl_3$); UV (methanol, $c = 4.4 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 209 (34200), 233 (13900) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3231$ (b, s), 2960 (s), 2926 (w), 2868 (m), 1661 (s), 1514 (s), 1258 (m), 864 (m), 785 (m), 737 (w) cm^{-1}; GC: single peak at $R_t = 10.1$ min; MS (70 eV): m/z (%) = 379 (22.9) [M^+], 331 (15.2), 330 (100.0), 315 (10.8), 286 (10.9), 252 (11.9), 160 (14.0), 144 (16.2), 130 (12.3), 118 (12.6), 91 (9.6), 77 (13.1).

N-[2-[[4-Iodo-2,6-bis(1-methylethyl)phenyl]amino]-2-oxoethyl]-N-(carboxymethyl)glycine (**1**, $C_{18}H_{25}IN_2O_5$)

A solution of 2.0 g of **1b** (5.3 mmol) in 140 cm^3 of ethanol was mixed with an equimolar amount of *IDA* (1.1 g) in 65 cm^3 of H_2O. The *pH* value of the reaction mixture was adjusted to about 10 by

addition of 1.25 g of Na_2CO_3. The reaction mixture was refluxed at 84–86°C during 10 h. Then it was cooled and filtered under vacuum. Ethanol was evaporated to leave an aqueous solution. The *pH* value of the aqueous solution was adjusted to 2.25, by adding 5 *M* HCl to precipitate **1**. After filteration **1** was dried and recrystallized from absolute ethanol to yield 1.14 g (45%). Mp 182–184°C; UV (methanol, $c = 4.2 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 209 (33000), 233 (13500) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3251$ (b, s), 3052 (b, w), 2961 (s), 2932 (w), 2870 (w), 1690 (s), 1542 (s), 1360 (s), 1330 (b, s), 892 (m), 865 (m), 847 (w), 706 (b, w), 680 (b, w) cm^{-1}; ^{1}H NMR (200 MHz, *DMSO*-d_6): $\delta = 9.518$ (s, NH), 7.461 (s, H-3, H-5), 3.571 (s, 4H, N–CH_2–CO), 3.516 (s, NHCO–CH_2–N), 2.990 (b, m, 2H, –$CH(CH_3)_2$), 1.111 (d, 12H, –CH_3) ppm; ^{13}C NMR (50 MHz, *DMSO*-d_6): $\delta = 173.177$ (COOH), 171.028 (CO–NH), 149.099 (C-3, C-5), 132.732, 132.259 (C-1, C-6), 94.372 (C-4), 58.179 (CO–CH_2–N), 55.648 (N–CH_2–COO), 28.258 (–CH<), 23.369 (($CH_3)_2$=) ppm.

4-Iodo-2,6-diethylbenzenamine (**2a**, $C_{10}H_{14}IN$)

Compound **2a** was prepared from 2,6-diethylbenzenamine in the same manner as **1a** was obtained from 2,6-bis(1-methylethyl)benzenamine. Yield 2.4 g (87%), yellowish-brown oily mass. The crude product was used in next synthetic step. TLC: $R_f = 0.36$ (*n*-hexane:benzene = 3:7), $R_f = 0.83$ ($CHCl_3$); UV (methanol, $c = 1 \cdot 10^{-4}$ mol dm^{-3}): λ_{max} (ε) = 211 (31100), 248 (17000), 292 (2900) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3482$ (w), 3399 (m), 2964 (s), 2932 (m), 2873 (m), 1619 (s), 1449 (s), 1376 (w), 1343 (w), 1283 (w), 1207 (w), 866 (m), 843 (w) cm^{-1}.

N-(4-Iodo-2,6-diethylphenyl)-2-chloroacetamide (**2b**, $C_{12}H_{15}ICINO$)

Compound **2b** was prepared from **2a** in the same manner as **1b** was obtained from **1a**. A 10 mmol run yielded 2.3 g (65%) of **2b**. Mp 191–192°C; TLC: $R_f = 0.10$ (*n*-hexane:benzene = 3:7), $R_f = 0.58$ ($CHCl_3$); UV (methanol, $c = 4.8 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 210 (34400), 235 (14200) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3235$ (b, s), 3028 (m), 2962 (s), 2925 (m), 2864 (m), 1665 (s), 1530 (s), 1456 (m), 1404 (m), 1327 (m), 1217 (s), 970 (m), 860 (s), 793 (s), 719 (m), 669 (m) cm^{-1}.

N-[2-[(4-Iodo-2,6-diethylphenyl)amino]-2-oxoethyl]-N-(carboxymethyl)glycine (**2**, $C_{16}H_{21}IN_2O_5$)

Compound **2** was prepared from **2b** in the same manner as **1** was obtained from **1b**. A 4.6 mmol run yielded 1.18 g (58%) of **2**. Mp 221–223°C; UV (methanol, $c = 3.7 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 209 (37200), 235 (15500) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3298$ (s), 3060 (b, m), 3012 (s), 2961 (s), 2876 (m), 1704 (s), 1665 (s), 1532 (s), 1408 (s), 1353 (s), 961 (s), 865 (s), 677 (s) cm^{-1}; ^{1}H NMR (200 MHz, *DMSO*-d_6): $\delta = 9.523$ (s, NH), 7.455 (s, H-3, H-5), 3.571 (s, 4H, N–CH_2–CO), 3.499 (s, NHCO–CH_2–N), 2.463 (q, $J = 7.6$ Hz, 4H, –CH_2), 1.063 (t, $J = 7.6$ Hz, 6H, –CH_3) ppm; ^{13}C NMR (50 MHz, *DMSO*-d_6): $\delta = 173.186$ (COOH), 170.564 (CO–NH), 144.466 (C-3, C-5), 135.008, 134.098 (C-1, C-6), 93.407 (C-4), 58.206 (CO–CH_2–N), 55.657 (N–CH_2–COO), 24.280 (–CH_2–), 14.621 (–CH_3) ppm.

2,4-Diiodo-6-methylbenzenamine (**3a**)

Compound **3a** was prepared from 2-methylbenzenamine in the same manner as **1a** was obtained from 2,6-bis(1-methylethyl)benzenamine, except that the molar ratio of 2-methylbenzenamine and *BTMA* ICl_2 was 1:2.1. A 20 mmol of 2-methyl benzenamine run yielded 6.25 g (87%) of **3a**, a light-brown crystal mass. Crystallization from *n*-hexane (58–60°C) afforded 5.46 g (76%) of **3a** as yellowish needles. Mp 78–79°C (Ref. [15] 80–81°C); TLC: $R_f = 0.60$ (n-hexane:benzene = 3:7), $R_f = 0.85$

($CHCl_3$); UV (methanol, $c = 4 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 220 (33400), 252 (15100), 303 (3200) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3397$ (b, s), 3303 (b, s), 1619 (s), 1456 (s), 1436 (m), 1053 (w), 852 (m), 716 (w), 651 (m), 541 (w) cm^{-1}; GC: single peak at $R_t = 8.01$ min; MS (70 eV): m/z (%) = 360 (8.32) [M + H$^+$], 359 (100.0) [M$^+$], 232.0 (22.26), 127 (12.41), 105 (28.97), 104 (28.74), 78 (11.59), 77 (10.60).

N-(2,4-Diiodo-6-methylphenyl)-2-chloroacetamide (**3b**, $C_9H_8I_2ClNO$)

Compound **3b** was prepared from **3a** in the same manner as **1b** was obtained from **1a**. A 14 mmol run yielded 4.45 g (73%) of **3b** as white crystals. Mp 223–224°C; TLC: $R_f = 0.12$ (*n*-hexane:benzene = 3:7), $R_f = 0.59$ ($CHCl_3$); UV (methanol, $c = 2.8 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 228 (30100) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3237$ (b, s), 3195 (b, s), 3016 (m), 1674 (s), 1572 (m), 1547 (w), 1519 (s), 1452 (m), 972 (w), 853 (m), 775 (m), 692 (m) cm^{-1}; GC: single peak at $R_t = 20.75$ min; MS (70 eV): m/z (%) = 435 (6), 358 (10), 350 (13), 310 (37), 309 (12), 308 (100), 272 (9), 271 (17), 231 (7), 181 (7), 104 (7).

N-[2-[(2,4-Diiodo-6-methylphenyl)amino]-2-oxoethyl]-N-(carboxymethyl)glycine (**3**, $C_{13}H_{14}I_2N_2O_5$)

Compound **3** was prepared from **3b** in the same manner as **1** was obtained from **1b**. A 9 mmol run yielded 2.9 g (61%) of **3** (crystallized from ethanol). Mp 224–225°C; UV (methanol, $c = 2.7 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 228 (30100) nm (mol^{-1} dm^3 cm^{-1}); IR (KBr): $\bar{\nu} = 3299$ (b, s), 3014 (s), 2965 (s), 1705 (s), 1674 (s), 1518 (s), 1417 (m), 1355 (m), 963 (m), 860 (m), 675 (m) cm^{-1}; ^{1}H NMR (200 MHz, *DMSO*-d$_6$): $\delta = 9.728$ (b, s, NH), 8.040 (s, H-3), 7.670 (s, H-5), 3.618 (s, 4H, N–CH_2–CO), 3.511 (s, NHCO–CH_2–N), 2.159 (s, –CH_3) ppm; ^{13}C NMR (50 MHz, *DMSO*-d$_6$): $\delta = 173.122$ (COOH), 169.799 (CO–NH), 143.638, 139.978, 139.077, 138.767 (C-1, C-3, C-5, C-6), 102.611 (C-2), 93.990 (C-4), 58.124 (CO–CH_2–N), 55.767 (N–CH_2–COO), 18.727 (–CH_3) ppm.

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