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Novel and facile methods for the synthesis of DTPA-mono-amide: a new completely revised strategy in radiopharmaceutical chemistry

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Abstract DTPA is a very strong metal chelator widely utilized in radiopharmaceutical chemistry for conjugation of chemicals which do not have enough potency for direct metalo-labeling and also to manage toxic radioactive materials such as plutonium, americium, and curium. It is difficult to conjugate DTPA to an amine group in a singular direction and such reactions usually also coincidently produce a mixture of DTPA-bis-amides and DTPA-mono-amide resulting in considerable insufficiencies/difficulties in synthesis and especially yield/separation procedures. In this paper, novel methods for the exclusive synthesis of DTPA-mono-amide have been established which extensively reduce the difficulties otherwise encountered and increase the reaction's yield considering the green chemistry approaches. This is expected to be of interest to radiopharmaceutical researchers interested in the DTPA (Radio)-metallic-conjugate. Overall, this paper provides a framework to achieve a higher degree of propriety from DTPA as a chelator to conjugate to the chemical compounds.

Keywords DTPA · DTPA-conjugate · Radiopharmaceutical chemistry · Sonication · Green chemistry

This research is a partial fulfillment of a PhD thesis in Radiopharmacy by Dr. Mehdi Shafiee Ardestani.

This study is also proudly dedicated to the living memory of Professor Iraj Bayat, "Father of Radiochemistry" in Iran.

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Abbreviations

DCC	Dicyclohexylcarbodiimide
DTPA	Diethylenetriamine-*N*,*N*,*N*′,*N*′,*N*″-pentaacetic acid
DTPA-DA	DTPA dianhydride
Gd^{3+}	Gadolinium(III)
ECFCA	Extracellular fluid complexing agent
EDC	*N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide
FDA	U.S. Food and Drug Administration
HOBT	Hydroxybenzotriazole
^{111}In	^{111}Indium
MRI	Magnetic resonance imaging
NHS	*N*-Hydroxysuccinamide
Prep-HPLC	Preparative high performance liquid chromatography
SPPS	Solid phase peptide synthesis
^{99m}Tc	Technetium
)))	Ultrasound irradiation

Introduction

Contrast-enhancing agents or chelator-bearing-radiometals are being increasingly used as novel tools in the diagnosis or treatment of diseases, and chelators play an important role in the imaging technology [1]. DTPA is an organic compound consisting of a diethylenetriamine backbone modified with five carboxymethyl groups. The molecule can be viewed as an elongated version of EDTA. DTPA is used as its conjugate base, having a high affinity for metal cations. Upon complex formation with lanthanide and actinide ions, DTPA exists as its pentaanionic form, i.e. all five carboxylic acid groups are deprotonated [1]. Since the 1960 s, physicians have used DTPA as a chelating agent to treat internal contamination from radioactive materials such as americium, plutonium, californium, and berkelium.

Currently, DTPA is approved by the FDA for chelation of only three radioactive materials: plutonium, americium, and curium. It is used as a soil extracting agent for available soil micronutrients such as Zn, Fe, Cu and Mn at the pH of 7.3 with calcium chloride and TEA. Furthermore, it is used to chelate gadolinium, a paramagnetic metal to form a MRI contrast agent, such as Gd^{3+}–DTPA (Magnevist®). Additionally, DTPA is widely used as an excellent ligand for radionuclides such as ^{111}In and ^{99m}Tc in radiopharmaceutical chemistry in order to label chemical compounds to be used in nuclear medicine or radiopharmaceutical chemistry studies [2–4]. DTPA is the parent acid of an octadentate ligand, diethylene triamine pentaacetate. In some situations, all five acetate arms do not attach to the metal ion. It has been used as a complexing agent to decontaminate humans who have been contaminated with actinides. Upon formation of chelate complexes, these heavy metal ions are poorly absorbed and are more readily eliminated in urine [1–4].

Chelation to the conjugate base of this ligand increases the solubility of Gd^{3+} at the body's neutral pH and still allows maintains the paramagnetic effect required as an MRI contrast agent. The $DTPA^{5-}$ ligand binds to Gd^{3+} through five oxygen atoms of the carboxylates and three nitrogen atoms of the amines [1, 2].

Magnetic resonance imaging (MRI) is a powerful and noninvasive diagnostic technique based on the differences between relaxation rates of water protons, and provides important graphical images of the inside of the human body. MRI contrast agents such as Gd–DTPA improve tissue discrimination in the MRI images [1, 2].

DTPA is classified as an ECFCA because of its reduced uptake by cells, and this is the main disadvantage of the compound. To increase DTPA cellular uptake several structural derivatives have been synthesized and examined in order to improve DTPA–metal complex formation and cellular uptake [5]. DTPA is also routinely used as a chelating agent to label chemical compounds indirectly in radiopharmaceutical chemistry and there are several examples given in the literature such as Vasovist, MS325 or B22956 (Fig. 1) [5].

There are some reported methods to conjugate DTPA to variety chemical compounds including different synthetic procedure to reach the final structure of DTPA-conjugate compound such as 1-(*p*-isothiocyanatobenzyl) ethylenediaminetetra acetic acid. Difficulties in the synthesis procedures of such compounds have limited their applications [6].

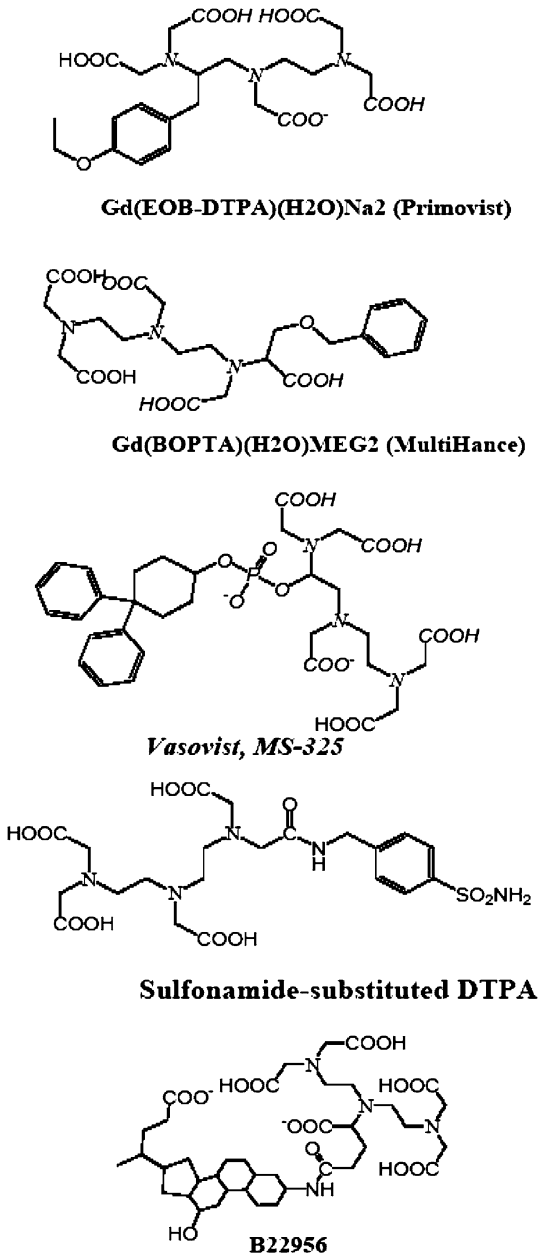


Fig. 1 Illustration of some DTPA conjugated compounds which labeled with Gadolinium

Another common and facile method to obtain DTPA-mono-amide is based on the synthesis of DTPA-DA from DTPA and acetic anhydride reaction in the presence of a base (Fig. 2) and subsequent reaction of DTPA-DA with amino-containing compounds and (1:1; mol/mol). Nevertheless, this approach is not as easy as it appears. DTPA-DA reacts with R-NH_2 and then produces a mixture of DTPA-mono-amide and DTPA-bis-amides (as the main product). The ample similarities between mono and bis amides DTPA structures result in separation difficulties, and therefore, classical methods are not suitable and often HPLC is required. The famous example pointed out in the literature is related to DTPA-Folate [7, 8]. DTPA-Folate was designed, synthesized and then labeled with ^{99m}Tc and ^{111}In for tumor imaging nuclear medicine studies and found fully successful [7, 8]. But in the reported synthesis procedure, DTPA-Folate and DTPA-di-Folate were both produced, and HPLC equipped with a preparative column was employed for separation. It is worthwhile mentioning that working with Prep-HPLC requires sufficient experiences, a qualified operator and utilizes a high cost and time while the amount of separation is very limited.

It should be noted that the reported yield of DTPA-mono-amide is low (usually less that 20%) [8–10]. There are some extensive attempts to solve and improve the yield of DTPA-monoamide synthesis, as briefly explained. In the more difficult methods as described in U.S. Patent No. 5021571 [11] or EP 263059 [12], DTPA-DA was synthesized and then made to react with the ethanol, Di-ethanol-DTPA, in the presence of NaOH/H^+. Afterwards, it was hydrolyzed to mono-side DTPA-Ethanol and the procedure continued until DTPA-ethanol-monoanhydride was produced. The next step was amine addition to DTPA-ethanol-monoanhydride in order to produce DTPA-monoamide-ethanol. Finally, going through these time-consuming stages, in the seventh step using NaOH/H^+, DTPA-monoamide was obtained, though with a low yield due to the saponification of ester groups. Other reports described the synthesis of DTPA-tetraesters [13, 14] in which compounds of general formula in Fig. 3 were produced by additional synthesis steps, in some cases more than five, and then amine was added to a carboxylic group and in the final step following the use of esteric saponification, DTPA-monoamide was obtained again with a low yield in this enlarged synthesis procedures.

Besides the above mentioned procedure, a new successful invention reported in the literature is based on the application of DTPA-DA in the presence of a catalytic amount of lithium salts or imidazole to increase solubility and reactivity and temperature over 50 °C. DTPA-monoamide was synthesized with a good yield of 40–50%, again in a mixture containing DTPA-monoamide and DTPA-diamides, thus requiring a separation procedure to be performed using Prep-HPLC [15]. In the present work, two novel procedures for DTPA-mono-amide synthesis with high yield and few reaction steps are described for the first time.

Experiment

All materials required for the study were provided from Sigma, USA without any further purification. Melting points were determined on a Kofler hot stage apparatus and are uncorrected (C. Reichert, Vienna, Austria). The FT-IR spectra were recorded on a Nicolet 550 instrument. ^{1}H and ^{13}C NMR spectra were measured on a Varian FT-400 unity plus (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer and also a Bruker 500 MHz spectrometer (Brucker, Rheinstetten, Germany) spectrometer with tetramethyl silane as an internal standard. Chemical shifts are given in δ. EIMS spectra were recorded on a Finnigan MAT EI TSQ-70 eV (Finnigan, USA) and also on Agilent Technology (HP), 5973 Network Mass. Silica gel 60 F254 precoated plates (Merck) were used for TLC. The purity of the synthesized compounds was confirmed by thin-layer chromatography (TLC) using various solvents of different polarities. Sonication was performed using a Sartorius Ultrasonic-Homogenizer LABSONIC®P230 V/50 Hz. The optimal conditions were set by sonotrode location under the liquid surface.

It should be pointed out that DTPA-DA is required for the start of all reactions and its synthesis procedure is described in Fig. 2. Briefly 7.6 mmol DTPA (3 g) was

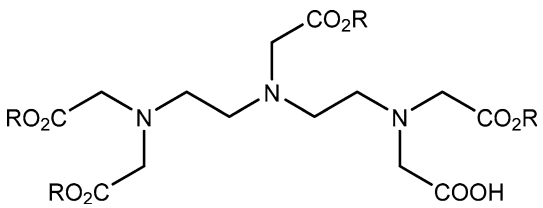


Fig. 3 DTPA-tetraesters, a precursor for DTPA-mono-amide synthesis

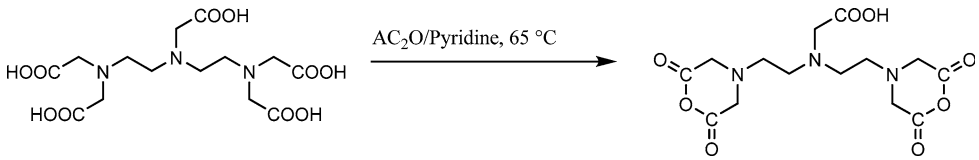


Fig. 2 The procedure for the synthesis of DTPA-Dianhydride (DTPA-DA)

dissolved in DMSO (10 mL), acetic anhydride (20 mL) and pyridine (3 mL) as base under anhydrous condition. The reaction was heated at 65 °C for 1 day. Subsequently, the reaction mixture was cooled, filtered and washed twice by acetic anhydride and anhydrous diethyl ether. The residue was dried to constant weight under vacuum (52 kPa) at 40 °C and yield a white powder with yield of 93%.

Two general procedures A and B were developed for the exclusive production of DTPA-mono-amide conjugate:

Procedure A

Sub-procedure A_1

1 mmol DTPA-DA was dissolved in anhydrous DMF and then 1.1 mmol DCC, 0.6 mmol HOBT, 0.2 mL triethylamine and 100 mg activated molecular sieve 3 Å were added to the media. The reaction mixture was stirred at room temperature for at least 35 min. Afterwards, the reaction temperature was elevated to 90–110 °C, and 0.333 mmol R-NH_2 was added to it, and the reaction mixture was heated (90–110 °C) for 24 h. TLC showed only one spot and no evidence of starting material. The reaction mixture was then cooled at room temperature (25 ± 1 °C), diluted with 0.2 mL water and 5 mL methanol and then filtered. A clear solution was collected and diethyl ether was added dropwise to the solution until a white DTPA-mono-amide crystallized and then filtered. The above steps were repeated for further purification until a yellowish-orange powder was obtained. The reaction outline and results are depicted in Fig. 4 and Table 1.

In case of DTPA-D-Deoxy-glucoseamine and/or compounds having molecular weights over 500D, innovate additional steps such as dialysis membrane (SPECTRUM®) application and water as purifying solvent, as a green chemistry approach, with a cut off of 100–500D were employed. This modification should be explored that in this study the dialysis bag was employed for easy and efficient purification of synthesized compounds having molecular weight over 500 from the reaction mixture. As shown in the original mass spectrum of DTPA-DG, as an example and evidence, which was purified by dialysis bag (cut off 100–500D, SPECTRUM®) in water medium illustrated in Fig. 5.

The molecular weight (Mw) of 715 (DTPA-$(DG)_2$) could not be observed in mass spectrum due to by proposed method only mono-amide of DTPA (DTPA-DG) produced not bis-amide.

Sub-procedure A_2

The above procedure was repeated except for the case of DCC which was replaced by 1 mmol EDC as carbodiimides. The results are presented in Table 1.

Sub-procedure $A_{3,4}$

The same materials and procedures as method $A_{1,2}$ were employed with the exception of the use of an ultrasonic irradiation with the following properties: minimum irradiation time was set at 20 min and little amounts of Silica as catalyst were employed in ultrasound irradiation. The results and outline of reaction are depicted in Table 1 and Fig. 4.

Procedure B

Sub-procedure B_1

1 mmol DTPA-DA was dissolved in 5 mL of different dried solvents, then 0.5 mmol triethylamine and activated molecular sieve 3 Å (100 mg) were added, temperature was raised to 90–95 °C, and meanwhile 0.5 mmol R-NH_2 was added and the mixture was stirred for 1 day. TLC [usually methanol:chloroform and in some cases ethyl acetate:hexane] confirmed only one spot and finally after purifications, spectroscopic data confirmed DTPA-mono-amide synthesis. Similar to method A, the reaction mixture was cooled at room temperature, filtered, evaporated by means of vacuum particularly and diluted with 0.2 mL water and 5 mL methanol. Meanwhile 10 mL diethyl ether was gently added and the resultant crystallized product (DTPA-mono-amide) was filtered and then dried to

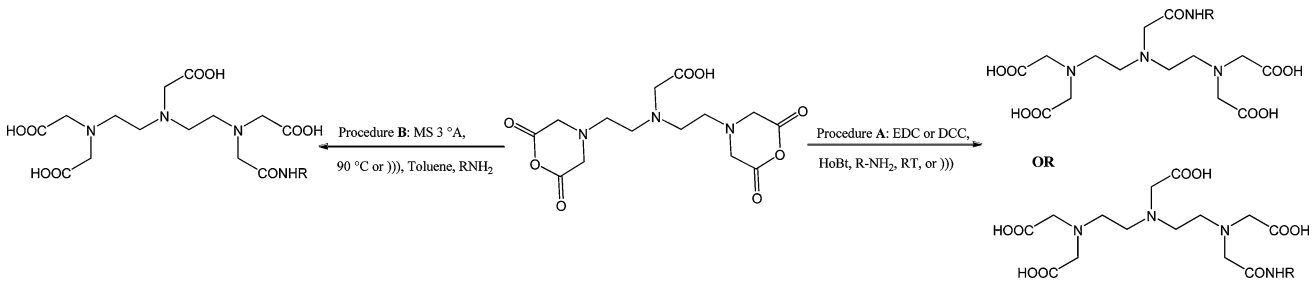
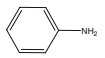
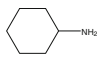
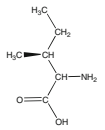
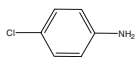
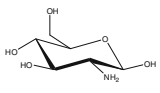
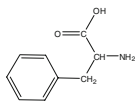
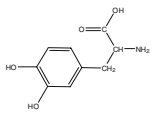
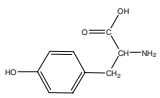
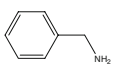
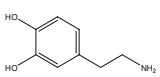


Fig. 4 Procedures A and B described for the exclusive synthesis of DTPA-mono-amide while using DCC or EDC or toluene/molecular sieve at presence or absence of ultrasound irradiation (see reaction time and yields in Table 1)

Table 1 Synthesis of DTPA-mono-amide: yields and reaction conditions based on the procedures A and B[a]

Amines	DTPA-mono-amide (%)						Reaction time					
	A				B		A_1 (h)	A_2 (h)	A_3 (min)	A_4 (min)	B_1 (day)	B_2 (min)
	A_1	A_2	A_3	A_4	B_1	B_2						
	46	52	64	73	62	78	5	4	5	5	1	10
	39	47	55	61	59	66	5	4	7	5	1	15
	33	43	46	55	46	60	8	8	10	10	1	15
	48	59	61	72	64	79	4	3	3	3	1	7
	40	48	56	68	56	71	24	24	30	15	1	30
	37	42	50	63	51	64	24	24	15	10	1	15
	33	36	45	51	40	55	24	24	20	10	1	15
	33	38	46	53	41	54	24	24	15	12	1	15
	40	45	55	65	48	70	12	10	10	10	1	20
	41	47	57	61	59	76	24	24	10	7	1	20

[a] Procedure A: A_1: DCC; A_2: EDC; A_3: DCC &))); A_4: EDC &))). Procedure B**:** B_1: MS Sieve 3 Å, 90 °C; B_2: MS Sieve 3 Å &))). For details see text

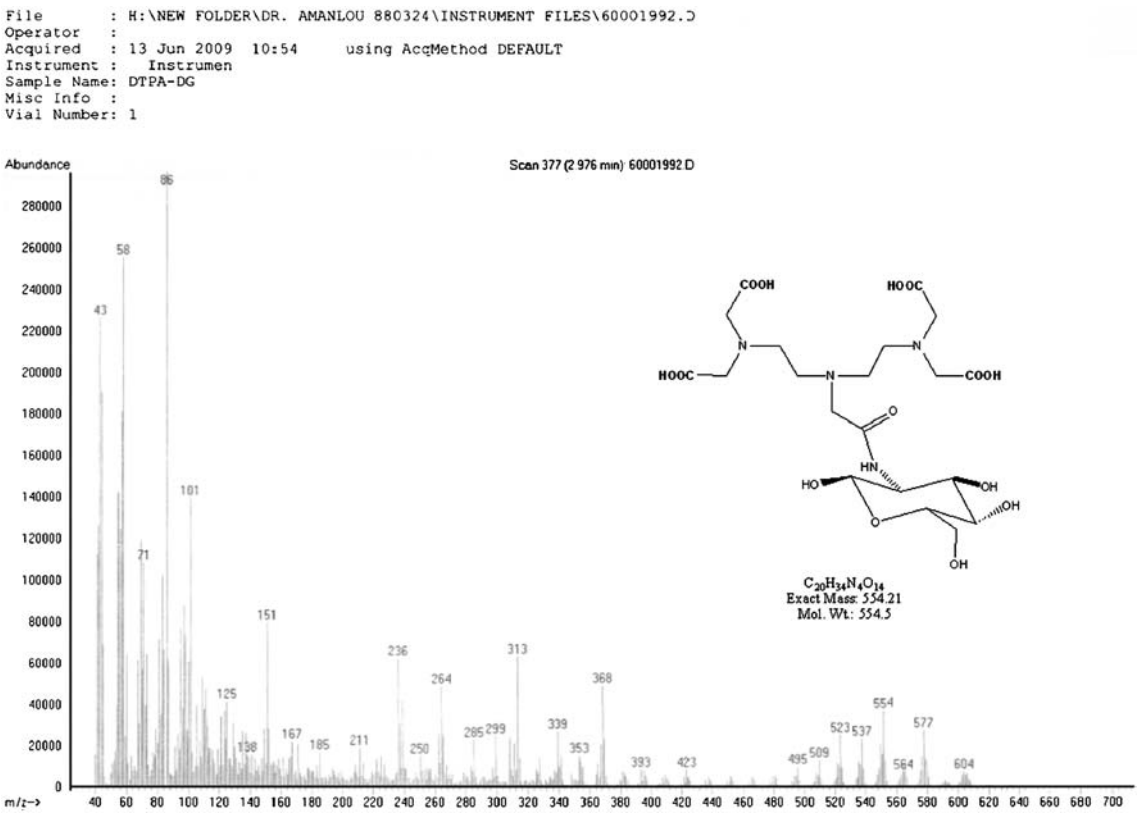


Fig. 5 The original spectrum of DTPA-DG which was purified by dialysis bag (Cut off 100–500D, SPECTRUM®). There is not any trace of DTPA-$(DG)_2$ (Mw = 715). The number 577 is related to Mw of $C_{20}H_{34}N_4NaO_{14}+$

constant weight. The results are shown in Table 1. The reaction outline was elaborately depicted in Fig. 4.

Sub-procedure B_2

A procedure similar to method B was employed with the only exception of the use of an ultrasonic environment with the following conditions: minimum irradiation time was set at 20 min and little amounts of silica as catalyst were employed in ultrasound irradiation. The results and outline of the reaction are depicted in Table 1 and Fig. 4. The amino compound was added to the reaction medium when the temperature was above 90 °C.

Spectroscopic data for the synthesized compounds

DTPA-DA

FT-IR (KBr, cm^{-1}): 1638.53, 1730.95, 1818.49, 2555.90, 2714.65 and 3103.84. ^1H NMR (500 MHz, DMSO): $\delta = 2.591$ (t, $J = 6.25$, 4H), 2.747 (t, $J = 6.25$, 4H), 3.299 (s, 2H), 3.704 (s, 8H), 11.012 (s, 1H). ^{13}C NMR (100 MHz, DMSO): 50.1, 59.2, 168.3, 174.1. MS: *m/z* %: 357.3.

DTPA-anilinamide

FT-IR (KBr, cm^{-1}): 695, 1655.21, 1723.02, 3350.63. ^1H NMR (500 MHz, DMSO): $\delta = 2.938$ (t, $J = 6$, 4H), 3.043 (t, $J = 6$, 4H), 3.448 (d, $J = 2.75$, 8H), 3.547 (s, 2H), 5.323 (s, 1H), 7.025 (t, $J = 8$, 1H), 7.264 (t, $J = 8$, 2H), 7.653 (d, $J = 4.25$, 2H), 11.053 (s, 4H). ^{13}C NMR (100 MHz, DMSO): 51.02, 52.28, 55.33, 58.25, 79.2, 95.43, 113.86, 119.37, 123.27, 128.65, 138.72, 169.34, 172.49, 173.02. MS: *m/z* %: 468.1.

DTPA-D-Deoxy-glucosamide (DTPA-DG)

D-glucosamine hydrochloride was neutralized using excess amounts of a base such as aqueous sodium bicarbonate. The reaction was stirred for at least 30 min, and then the reaction mixture was filtered, and ascorbic acid or sodium metabisulfate as an antioxidant agent was added into the solution. Immediately and without any hesitation, the solution was spray dried or lyophilized and the mild yellowish powder (D-glucosamine, base) was obtained with a yield of 97%. DTPA-D-Deoxy-glucosamine was purified using the dialysis bag with a cut off of 500D in a medium of water during within 24 h. The remained solution was

removed from the dialysis bag and then lyophilized. An extra pure white powder was obtained. This compound was synthesized previously with a yield of 38% suffering from HPLC purification [16]. Based on our results, we were able to obtain a yield of 40–71%. UV λ_{max}: 273 nm. FT-IR (KBr, cm^{-1}): 1070.84, 1203.98, 1460.03, 1588.05, 1618.78, 1746.80, 2924.61, 3329.16, 3426.46. ^{1}H NMR (500 MHz, DMSO): $\delta = 1.173$ (s, 4H), 1.506 (d, $J = 4.75$, 4H), 1.991 (t, $J = 6$, 4H), 2.684 (s, 8H), 2.863 (s, 2H), 3.183 (d, $J = 4$, 1H), 3.442 (d, $J = 4$, 1H), 3.571–3.622 (m, 3H), 4.435 (d, $J = 4$, 1H), 5.382 (d, $J = 4$, 1H), 8.171 (NH-amide, 1H), 12.273 (S, 4H). ^{13}C NMR (100 MHz, DMSO): 50.21, 58.78, 64.2, 75.1, 76.02, 78.21, 47.61, 94.31, 169.82, 173.21, 175.1, 174.03. MS: *m/z* %: 554.3.

DTPA-N-benzylamide

FT-IR (KBr, cm^{-1}): 1555.33, 1643.21, 3302.31. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.921$ (t, $J = 6$, 4H), 3.101 (t, $J = 6$, 4H), 3.432 (d, $J = 3$, 8H), 3.547 (s, 2H), 5.401 (s, 2H), 6.241 (s, 1H), 7.214–7.326 (m, 5H), 12.123 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 43.4, 127.2, 127.6, 128.5, 139.1, 172.8. MS: *m/z* %: 474.2.

DTPA-cyclohexylamide

FT-IR (KBr, cm^{-1}): 770.12, 1037.03, 1639.31, 1735.06, 2921.23, 2938.11, 3368.33. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.301$ (d, $J = 6$, 4H), 1.483 (m, 1H), 1.912 (m, 4H), 2.432 (t, $J = 6$, 4H), 2.747 (t, $J = 6$, 4H), 3.181 (s, 2H), 3.653 (s, 8H), 6.553 (d, $J = 3.5$, 1H), 11.222 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 22.5, 28.3, 47.3, 34.1, 57.2, 50.2, 173.2, 170.5. MS: *m/z* %: 474.2.

DTPA-phenylalaninamide

FT-IR (KBr, cm^{-1}): 1646.78, 1732.53, 3353.44. ^{1}H NMR (400 MHz, DMSO): $\delta = 2.948$ (m, 4H), 3.044 (t, $J = 7$, 4H), 3.260 (d, $J = 5.75$, 8H), 3.465 (s, 2H), 3.582 (s, 1H), 4.450 (m, 2H), 7.179–7.301 (m, 5H), 8.251 (d, $J = 1.5$, 1H), 11.334 (s, 5H). ^{13}C NMR (100 MHz, DMSO): 36.2, 51.1, 55.2, 58.1, 126.6, 127.1, 128.4, 169.3, 172.7. MS: *m/z* %: 540.3.

DTPA-tyrosinamide

FT-IR (KBr, cm^{-1}): 1659.21, 1757.98, 3242.31, 3368.33. ^{1}H NMR (400 MHz, DMSO): $\delta = 2.023$ (t, $J = 6$, 4H), 2.144 (t, $J = 6$, 4H), 3.228 (s, 8H), 3.323 (s, 2H), 3.587 (m, 2H) 4.093 (s, 1H), 6.982 (m, 2H), 7.134 (m, 2H), 7.725 (s, 1H) 8.198 (s, 1H), 11.223 (s, 5H). ^{13}C NMR (100 MHz, DMSO): 36.6, 49.7, 51.2, 57.8, 58.1, 129.3, 131.8, 170.6, 174.5. MS: *m/z* %: 556.3.

DTPA-dihydroxyphenylalaninamide

FT-IR (KBr, cm^{-1}): 1666.22, 1752.87, 3463.94. ^{1}H NMR (400 MHz, DMSO): $\delta = 1.848$ (m, 4H), 2.144 (t, $J = 6$, 4H), 3.337 (s, 8H), 3.413 (s, 2H), 3.587 (m, 2H) 4.093 (s, 1H), 6.439–6.621 (m, 3H), 8.485 (s, 1H), 8.641 (m, 2H), 11.934 (s, 5H). ^{13}C NMR (100 MHz, DMSO): 35.1, 51.1, 55.2, 58.1, 127.1, 128.2, 170.6, 171.7. MS: *m/z* %: 572.2.

DTPA-dopamineamide

FT-IR (KBr, cm^{-1}): 1603.34, 1725.74, 3310.11, 3431.78. ^{1}H NMR (500 MHz, DMSO): $\delta = 2.644$ (t, $J = 6$, 4H), 2.793 (t, $J = 6$, 4H), 2.834 (m, 2H), 3.215 (s, 8H), 3.387 (s, 2H), 4.122 (s, 1H), 6.967 (m, 1H), 7.233 (m, 2H), 7.746 (s, 2H), 8.332 (s, 1H), 11.155 (s, 5H). ^{13}C NMR (100 MHz, DMSO): 36.1, 40.5, 50.1, 57.8, 170.2, 173.1. MS: *m/z* %: 528.2.

DTPA-pentanoic-amide

FT-IR (KBr, cm^{-1}): 1665.76, 1745.33, 3423.92. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.994$ (t, $J = 2.5$, 3H), 1.112 (S, 3H), 1.302 (m, 2H), 2.401 (t, $J = 6$, 4H), 2.704 (m, 1H), 2.819 (t, $J = 6$, 4H), 3.202 (s, 2H), 3.653 (s, 8H), 4.214 (m, 1H), 6.671 (d, $J = 3.25$, 1H), 11.802 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): 12.1 15.4, 50.2, 55.1, 24.8, 35.8, 57.4, 174.1, 173.1. MS: *m/z* %: 506.2.

Results and discussion

The main criteria in applied methods are based on the following concepts:

1. After the primary and pilot studies, we were surprised to find that DTPA acted peculiarly in substituted nucleophilic (amidation) reaction type-2 (SN-2): what this means is that in room temperature, DTPA tends to produce di-amide, and in temperatures over 50 °C, it shows more potency for mono-amide production. In repeated reactions, TLC evidences showed two spots related to the mono and bis-amides-DTPA. Moreover, increasing the temperature from 50 to 75 °C decreased the amount of DTPA-bis-amides production. It seems to us that from a thermodynamic perspective, the reaction in room temperature tends to produce di-amides (as major product) as well as mono-amide as minor product; however in high temperatures, over 75 °C, we found mostly DTPA-monoamide as major product.
2. DTPA amidation reaction is very sensitive to the presence of H_2O (from the solvent or amidation product). In media containing water, activated carboxylic groups will be rapidly inactivated. Two strategies

should be considered; use of a) dried reaction medium and b) hydroscopic agent such as activated molecular sieves. These conditions help to increase the yield.

3. The use of molecular sieves has shown interesting effects in organic chemistry reactions such as transesterification of ethyl acetoacetate with higher alcohols without using any catalysts. The mentioned reaction was performed in the presence of activated molecular sieves and toluene only [17] or amidation reaction [18]. It was reported that molecular sieves only trap the released water in amidation reaction [18]. In this manner, activated molecular sieves and different kinds of solvents were employed together. The observations showed 100% successful results toluene-only as the solvent of choice. It seems that molecular sieves and toluene can form an azeotropic system which removes produced H_2O more efficiently, and this increases the amount of amidation reaction while molecular sieves can absorb H_2O. According to previous reports, molecular sieves showed an ability to absorb alcohols [19], and therefore there exists the possibility for R-NH_2 absorption as well as those of alcohols [19]; this may statistically decrease the chance of two-side-amide formation but needs to be further investigated.
4. The temperature of choice for the reactions was set at >90 °C because high temperature assists the reaction thermodynamically to improve in terms of DTPA-mono-amide yield.
5. According to statistical principles, changing the number of active sites in DTPA from two to three and the use of 1 mol DTPA having three active sites to 0.333 mol R-NH_2, the possibility of DTPA-mono-amide production is statistically increased, and if the reflux at the temperature above 90 °C is added, the probability of the reaction will be rationally intended to produce DTPA-mono-amide rather than before.
6. Reports suggest the important role of the ultrasonic irradiation to shorten the time and increasing the yield of reactions in organic chemistry, and thus we set to perform our synthesis procedures once again in the presence of the ultrasound. Compared with classical methods, this technique is more convenient, easily controlled and can have a very high potential in green chemistry approach [20–22]. It should be notified that amino conjugating region at DTPA is not important, while the only important fact is producing DTPA-mono-amide hence the difference in the DTPA-region of conjugation to amino compound does not produce any complications in its applications such as metal-complex formation or cell uptake entrance [8–15]. On the other hand, the binding site of the targeted amino compound is very important; for example in case of D-glucosamine, the binding sites are only limited to carbon numbers 1 and 2 since other carbons have an important role in cell uptake, and metabolism of the hexose and conjugation at the above sites may produce complications [23].

The next major portion of the study was related to reordering DTPA for conjugation due to the reported facilities rather than before in the laboratories and this is our anticipation. We have used a rationale strategy about DTPA-mono-amide synthesis when employing a statistically accepted principle; this means that we increased the number of active sites in DTPA for SN_2 type reaction and then quantitatively used an amount of R-NH_2 that only reacted with one of itself. For the activation of the only remaining carboxylic group in DTPA, different types of carbodiimide family were employed. The results suggest higher yields for EDC compared to DCC. Carbodiimide derivatives are being increasingly used in carbonyl group activation due to more ease of use and higher safety, for a given yield, compared to previous established methods, such as the use of thionyl chloride.

EDC is more resistant to H_2O resulting from the amide bond production or to reaction media than DCC and this may be an important point in explaining a higher yield for EDC compared to that of DCC in amide bond production [24–26]. It should be pointed out that the use of EDC as in the literature shows high reaction yields comparing to that of DCC [24–26]. Nevertheless, some difficulties such as high cost or extreme precision in storage have limited the use of EDC in comparison to DCC. Finally, it is worth noting that activation of the carbonyl group by DCC or EDC is more reactive than that of the anhydride group in SN-2 reaction, and we have used this point in the synthesis of DTPA-mono-amide in addition to the use of temperatures over 90 °C which lowered the chance of DTPA-bis-amide synthesis as a general and critical point of view. In general, DCC and DCC-related derivatives discussed previously form an asymmetrical anhydride. The anhydrides are usually very reactive and have been used extensively in SPPS especially in Boc synthesis [24–26]. In DCC/EDC mediated organic chemistry reactions, additives such as HOBT or NHS are often added to increase yields and decrease side reactions [24–26]. In all reactions the absence of molecular sieve caused fail in the production of DTPA-mono-amide. So presence of activated molecular sieve is a major principle for the synthesis of DTPA-mono-amide. In some reports in the literature [18, 19], it has been suggested that molecular sieves can adsorb excess of alcohol from the media, while we propose that molecular sieves may adsorb the amine group which is more nucleophilic than alcohol. Consequently, our reactions, within this assumed framework, went in the direction that we expected (i.e. DTPA joined with an amine in a singular

direction). In addition, the presence of toluene makes an azeotrope [18] and removes water formed from the reaction media and increases the yield. Finally the use of temperature over 90 °C further assists the molecular sieves and toluene in the production of DTPA-mono-amide.

Conclusions

The results demonstrate two novel and facile approaches to synthesize DTPA-mono-amide with high yields and involving decreased reaction times compared to previously reports results [8–12]. It is worth noting that these methods are sufficient even for upscaling DTPA-mono-amide synthesis. Furthermore, the use of ultrasonic environment decreases the reaction time and increases the yield more than those in the absence of ultrasonication. According to the obtained results, we established new facile ways to link DTPA as a well-known chelator to the amino group within the framework of green chemistry approaches such as the use of dialysis bag and purification in a medium of water or ultrasound irradiation. Shortening the reaction time and increasing the yield are results that can decrease the cost of the synthesis procedure compared to other approaches as reported in the literature [8–15].

Facile synthesis of DTPA-mono-amide can be a valuable tool for scientists in the tasks of labeling or preparation of kits. For example it is well established that DTPA cannot enter cells [8–12] in the human body so if an amine such as diethyl amine or D-glucosamine with cell uptake ability peculiarly is conjugated to DTPA, the resultant compounds will have the capability of cell entrance and may be used to treat the intracellular radio-metal overload, or in the context of radiopharmaceutical labeling, having the ability of D-glucosamine to penetrate cancer cells. The complex radio-metal-DTPA-D-glucosamine may be used for the diagnostic or treatment of viable cancer tumors as a novel selective strategy in oncology, which we are also actively pursuing. Here we have shown the ability of DTPA-D-glucosamine to form a complex with a metal. The above statements indicate particularly the importance of DTPA conjugation and its possible applications.

In this study we used a number of strategies as recently outlined in the literature, such as using an alternative base as suggested by others [26]. In a very interesting report, Zhang et al. used microwave irradiation as a green chemistry approach in the amidation reaction, which is very close to the use of ultrasound irradiation [27]. The results show a very important role of molecular sieves in the amidation reactions.

According to the Lipinski's Rule of Five, increasing the molecular weight decreases the cell uptake or binding ability of the ligand to the targeted receptors, therefore, DTPA-mono-amide is more efficient than DTPA-bis-amides [28]. In addition, the chelating potency of DTPA in DTPA-mono-amide seems to be more than DTPA-bis-amides. Hence, production of DTPA-mono-amide has more preferentially than DTPA-bis-amides due to lower molecular weight than DTPA-bis-amide.

Upon appropriate experience and familiarity with the reaction method proposed in this work, it would take one to 2 h to get a bulk of pure compound, while classical methods, in our experience, took at least 1 day while reports in the literature state even a longer time to achieve the reported results.

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