Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 19 Nov 2007.

To cite this article: Gülşen Öztürk, Bahattin Gümgüm, Murat Kizil & Sevil Emen (2007) Solvent-Free Synthesis of Nitrilotriacetamide and Diketopiperazines from Nitrilotriacetic Acid under Microwave Irradiation and Their Antimicrobial Activity, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 37:22, 3981-3988, DOI: 10.1080/00397910701572795

To link to this article: http://dx.doi.org/10.1080/00397910701572795

Synthetic Communications®, 37: 3981–3988, 2007

ISSN 0039-7911 print/1532-2432 online
DOI: 10.1080/00397910701572795

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Abstract: Efficient synthesis of dioxopiperazines-diketopiperzines (DKPs)- and amide from nitrilotriacetic acid (NTA) using microwave irradiation and classical heating were described. All compounds were characterized using ^{1}H NMR, ^{13}C NMR and elemental analysis. Antimicrobial effects of these compounds are also investigated. All tested compounds showed moderate antimicrobial activity.

Keywords: amide, antimicrobial activity, diketopiperazine, microwave irradiation, solvent-free synthesis

The formation of the amide bond by the direct combination of carboxylic acids and amines is highly desired, as the methods employed may also be utilized in a peptide and lactam synthesis. In general, the formation of carboxyamides from amines and carboxylic acids implies the activation of the carboxyl groups.[1,2] Nitrilotriacetamide is an ubiquitous tripodal ligand for metal coordination, but there are very few reports of nitrilotriacetamide in the literature.[3,4] An efficient method to prepare 2,6-diketopiperazine (DKP)

Received in the U.K. December 1, 2006
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involves the tandem formation of the N_1-C_2 and N_4-C_5 bonds. The intramolecular N-acylation reaction to form the N_1-C_2 bond is a common method of 2,6-DKP synthesis. The acyclic precursor generally contains a primary or secondary amide, frequently derived from a nitrile, as the nucleophilic nitrogen source and an acid as the acylating group. An early synthesis by Cignarella illustrates the efficiency with which one can construct the 2,6-DKP ring system by the cyclization of an amide onto an ester group. The acid bromide was sequentially coupled to an amine and an α-amino ester to give amide, which was simply heated to provide 2,6-DKP in good yield.[5]

Previously, D'Angeli and coworkers reported the treatment of α-bromocarboxamide with sodium hydride to produce the hexasubstituted 2,6-DKP. Early reports focused on elevated dehydration of diacids in the presence of various nitrogen sources, such as urea,[6] ammonia,[7] ammonium format,[8] and a primary amine.[9] 1-Aryl-2,6-DKPs was prepared by Li et al.[10] and evaluated for antitumor activity in a report by Abdel-Hamide directed toward the synthesis of new antimicrobial agents.[11] DKPs remain important in drug discovery because they contain constrained amino acids imbedded within their structures, without the unwanted physical and metabolic properties of peptides. The regioisomeric dioxopiperzines are found in numerous natural and synthetic organic compounds, and this substance possesses a multitude of important biological properties.[12–18] Nonetheless, dioxopiperazines of this class exhibit synergistic antitumor effects[19,20] in combination with clinically efficacious antineoplastic drug.

Research on the synthesis of ether, ester, etc., using microwave irradiation is going on in our laboratory.[21–23] In the present study, to avoid the preliminary and often expensive synthesis, pyrolitic preparation of amides as method for their synthesis in the absence of any catalyst and solvent was considered. Therefore, we aimed to simplify the well-known procedure [Eq. (1)] of amide synthesis from carboxylic acid and amine reaction by microwave application.

GENERAL PROCEDURE FOR SYNTHESIS OF AMIDE AND DIKETOPIPERAZINES

All chemicals were reagent grade unless otherwise specified. Amines and nitrilotriacetic acid were purchased from Fluka Chemical Company. The START labstation (Milestone labstation for microwave enhanced chemistry) was used. Silica gel/TLC cards (F254) were used for thin-layer chromatography (TLC). Melting points were determined with a Gallenkamp apparatus with open capillaries. Infrared spectra were recorded on a Mattson 1000 FTIR model spectrophotometer. Elemental analyses were obtained with a Carlo-Erba 1108 model apparatus. ^{1}H (400-MHz) and ^{13}C (100-MHz) NMR spectra were recorded on a Bruker DPX-400 high-performance digital FT-NMR spectrometer.

Nitrilotriacetic acid (0.50 g, 2.62 mmol) and amine (1.25 g, 11.70 mmol) were taken in the reaction vessel. The resultant mixture was heated at 150°C

with microwave irradiation and conventional heating. The mixture was then cooled and extracted with chloroform. The extract was washed successively with solution of 2 M HCl (50 mL) and a solution of 5% $NaHCO_3$ (50 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was crystallized from a mixture of dichloromethane and hexane.

SYNTHESIZED COMPOUNDS

2,2′,2′-Nitrilotris (N-Benzyl Acetamide) (1a)

Mp 143–144°C (lit. mp = 145),[24] ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.25 (s, 2H), 4.30 (d, 2H, $J = 5.90$ Hz), 7.21–7.31 (m, 5H), 7.70 (broad, 1H); ^{13}C NMR (100 Hz, $CDCl_3$): δ 43.38, 60.42, 127.52, 127.71, 128,71, 137.97, 170.17; IR (KBr): 3294 (NH), 1655 (C═O), 1537 (Ar-H), 1074 (C-N). Anal. calcd. for $C_{27}N_4H_{30}O_3$: C, 70.74; N, 12.23; H, 6.55. Found: C, 72.25; N, 11.93; H, 6.88.

N-Dibenzyl-2-(4-dibenzyl-3,5-dioxopiperazine-1-yl)acetamide (1b)

Mp 144–145°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.20 (s, 2H), 3.50 (s, 4H), 4.50 (d, 2H, $J = 8.10$ Hz), 5.02 (s, 2H), 7.03 (broad, 1H), 7.25–7.39 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 42.48, 43.26, 56.79, 59.35, 77.03, 77.35, 127.77, 129.00, 136.29, 137.76, 167.55, 168.69; IR (KBr): 3321 (NH), 1695 (C═O), 1663 (C═O), 1537 (Ph), 1074 (C-N). Anal. calcd. for $C_{20}N_3H_{21}O_3$: C, 68.37; N, 11.96; H, 5.98. Found: C, 68.70; N, 11.27; H, 6.00.

N-Hexzyl-2-(4-hexzyl-3,5-dioxopiperazine-1-yl)acetamide (2)

Mp 177–178°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, 6H, $J = 0.94$ Hz), 1.31 (m, 8H), 1.65 (m, 8H), 3.21 (m, 4H), 3.30 (s, 2H), 3.50 (s, 4H), 6.66 (broad, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.97, 22.49, 26.56, 27.82, 29.55, 31.37, 38.25, 39.42, 56.87, 59.48, 167.66, 168.80; IR (KBr): 3281 (NH), 1694 (C═O), 1576 (C═O), 1537, 1054 (C-N).

N-Buthyl-2-(4-buthyl-3,5-dioxopiperazine-1-yl)acetamide (3)

Mp 179–180°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, 6H, $J = 16.60$ Hz), 1.32 (m, 4H), 1.65 (m, 4H), 3.20 (m, 4H,), 3.30 (s, 2H), 3.51 (s, 4H), 6.66 (broad, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.07, 19.97, 20.03, 29.85, 29.55, 31.52, 38.55, 38.65, 54.92, 57.50, 166.87, 168.72.

TEST MICROORGANISMS

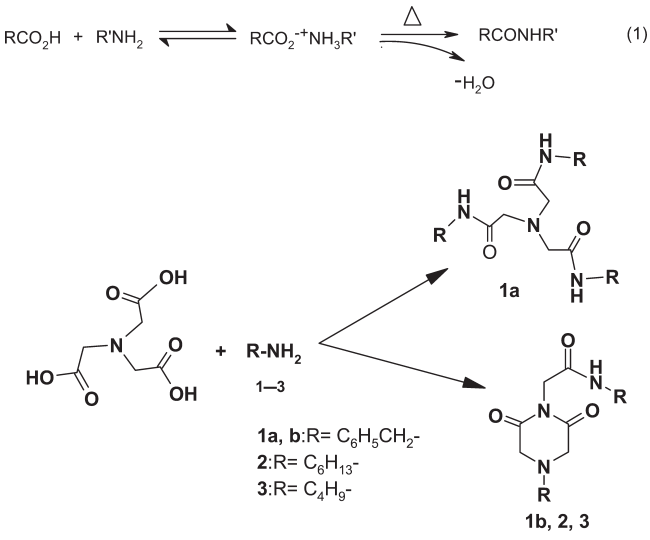
The in vitro antimicrobial activities of amide (**1a**) and diketopiperazines (**1b–3**) were tested against laboratory control strains belonging to the American Type Culture Collection (Maryland, USA): *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028), except for *Klebsiella oxyloca, Basillus cereus*, *Streptecocus pyogenes*, and *Candida albicans*, which were obtained from the Microbiology Department at the University of Dicle (Diyarbakır, Turkey).

EVALUATION OF ANTIMICROBIAL ACTIVITY

Antimicrobial activity was assayed by the disc diffusion susceptibility test according to the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS).[25] The disk diffusion test was performed on Muller–Hinton agar plates. Plates were dried at 35 to 36°C for about 30 min in an incubator before inoculation. Three to five freshly grown colonies of bacterial strains were inoculated into 25 mL of Mullar–Hinton broth medium in a shaking water bath for 4 to 6 h until a turbidity of 0.5 McFarland (1×10^8 CFU/mL) was reached. Final inocula were adjusted to 5×10^5 CFU/mL. Three to five colonies of *C. albicans* were inoculated into 25 mL of Sabouraud dextrose broth in a shaking water bath for 8 to 10 h until a turbidity of 0.5 McFarland was reached. The final inocula were adjusted to 5×10^5 CFU/mL using a spectrophotometer.[26] The inoculum (50 μL) from the final inocula was applied to each agar plate and uniformly spread with a sterilized cotton spreader over the surface. Absorption of excess moisture was allowed to occur for 30 min before application of sterile filter-paper discs. Sterile filter-paper disks (Oxoid, England, 6 mm in diameter) were impregnated with 20 μL of the sample solutions in dimethylsulphoxide (DMSO), 1.5 mg per 1 mL of DMSO (all solutions were filter-sterilized using a 0.20 μm membrane filter) and placed on inoculated plates. These plates were incubated at 37°C for 24 h for bacteria and 48 h for fungi. Standard disks of Amoxycillin/clavulanic acid (AMC, 30 μg/disc), and imipenem (IMP, 10 μg/disc) were individually used as positive controls, and the disks imbued with 20 μL of pure DMSO were a negative control. The diameters of the inhibition zones were measured in millimeters using an inhibition zone ruler.

RESULTS AND DISCUSSION

The reactions of carboxylic acid with amines were carried out without catalyst and solvent. Reaction temperature was around 150°C to favor shifting of equilibrium by water removal (Eq. 1). To compare the efficiency of



Scheme 1. Synthesis of amide and diketopiperazines from NTA and amines.

microwave heating with conventional heating, amide 2,6-DKP derivatives (NTA) tricarboxylic acid and amines were synthesized by both methods (Scheme 1). Compound **1a** was obtained by excess of amine within 45 min under microwave irradiation with excellent yield (94%) and conventional heating at the same time with low yield (less than 5%), but **1b** was obtained within 15 min under microwave irradiation with an excess of nitrilotriacetic acid (NTA) with good yield (80%) and conventional heating at the same time with low yield (less than 5%) (Table1). Tang et al. have synthesized **1a** using ethyl ester of nitrilotriacetic acid with benzylamine at 120–125°C within 12 h with 50% yield.[27] DKP derivatives were synthesized under microwave irradiation in a very short time without solvent or reagent at one step. In contrast, 2,6-DKP derivative was reported from dicarboxylic acid–amine with three steps using DCC (dicyclohexylcarbodiimide) in THF in 2 h with 43% yield by Prakash et al. to prepare a neuroleptic agent.[13] This clearly shows that our method is very efficient for the synthesis of amides.

Diketopiperazines have been repeatedly shown to exhibit strong biological activities.[28–30] Amide (**1a**) and diketopiperazines (**1b–3**) showed moderate antibacterial activity and antifungal activity (Table 2). Diketopiperazines 2 indicated 14-mm inhibition zones against *Escherichia coli* (ATCC 25922) and 13-mm zones against *Candida albicans*. Diketopiperazines **1b**, **2**, and **3** showed the same inhibition zones (12 mm) against *Streptecocus pyogenes*. Compound **1a** and **1b** indicate the same inhibition zones (10, 8, 10, and 8 mm against *Escherichia coli* (ATCC 25922), *Bacillus cereus*, *Klebsiella*

Table 1. Optimization of the reaction condition of microwave-assisted (600 W) and conventional amide and DKP formation [nitrilotriacetic acid (NTA)/amine]

Compound	Relative amount (mmol) NTA/amine	Reaction temp. (°C)	Reaction time (min)	Yield[a] (%)	
				MW	Δ
1a	2.6/11.7	150	15	76	+
1a	2.6/13.4	150	45	94	+
1a	2.6/7.8	150	60	NT	8
1a	2.6/7.8	180	20	NT	41
1a	2.6/9.3	180	60	NT	61
1a	2.6/7.8	180	60	NT	59
1a	2.6/18.2	180	60	NT	41
1b	4.2/7.3	150	15	80	+
1b	2.6/7.3	150	15	50	—
1b	2.6/2.6	150	15	34	—
1b	2.6/3.6	180	60	NT	16
1b	2.6/5.4	150	20	50	—
2	2.6/12.6	150	30	7	—
2	2.6/8.7	150	15	11	—
2	2.6/7.8	200	60	NT	12
3	2.6/10	150	20	7	—
3	2.6/7.8	200	60	NT	13

[a]In isolated products.
−: no reaction.
+: low yield (less than 5%).
NT: not tested.

Table 2. Antimicrobial activity of amide (**1a**,**b**) and diketopiperazines (**1b**,**2**,**3**) and standard antibiotics

	Zones of inhibition (mm)					
	Amide and diketopiperazines (20 μL/paper disc)				Standard antibiotics (μg/paper disc)	
Tested organisms	**1a**	**1b**	**2**	**3**	AMC 30	IPM 10
Escherichia coli, ATCC 25922	10	10	14	8	20	28
Salmonella typhimurium, ATCC 14028	10	8	12	—	24	26
Streptecocus pyogenes[a]	10	12	12	12	>30	>30
Bacillus cereus[a]	8	8	12	8	16	30
Klebsiella oxyloca[a]	10	10	12	—	18	26
Candida albicans[a]	8	8	13	8	NT	NT

[a]Clinical isolates.
Notes. IPM: imipenem; AMC: amoxycillin/clavulanic acid; NT: not tested; —: not active.

oxyloca, and *Candida albicans* respectively. The antimicrobial activity of the diketopiperazines **2** was found to be similar to amoxycillin against *Bacillus cereus*. All organisms are susceptible to imipenem. The DMSO (20 μL) negative control showed no inhibiting effect.

The synthesis of nitrilotriacetamide and diketopiperazines using microwave irradiation was achieved for the first time by our group. Nitrilotriacetamide and diketopiperazines were synthesized in very short time and without any reagent and solvent. All tested compounds were found to have moderate antimicrobial activity.

ACKNOWLEDGMENT

Financial support by University of Dicle (grant no: DÜAPK 04-FF-51) is gratefully acknowledged.

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