Monatshefte für Chemie 131, 937–943 (2000)

Monatshefte für Chemie
Chemical Monthly

Microwave-Assisted Solid-Phase Synthesis of Cephalosporin Derivatives with Antibacterial Activity

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Summary. The reaction of heterocyclic acids with 7-amino-cephalosporanic acid adsorbed on basic alumina under microwave irradiation afforded the N-acylated cephalosporin analogues in satisfactory yield. All compounds were tested for their antibacterial activity; some of them showed significant antibacterial properties. Cefotaxime and cephalothin were used as reference drugs.

Keywords. 7-Aminocephalosporanic acid; Solid support; Microwave irradiation; Antibacterial activity.

Introduction

The discovery of cephalosporin C, the first example of a naturally occurring cephalosporin [1], was the beginning of a new era in the search for effective antibacterials containing the β-lactam moiety. Since then many advances have been made in the synthesis, chemical modification, and biology of these molecules [2]. The increased chemical and biological stability of 7-substituted analogs (cephalothin, cefotaxime) illustrates the advantages of structurally modified derivatives.

A literature review shows that most of the synthetic strategies have been patented. Coupling of 7-*ACA* (**2**) with an acid in the presence of triethyl amine requires a reaction time of more than 4 hours [3]; a modification using $SO_3 \cdot DMF$ [4] at pH 7.5–8.0 still needs 2 hours. Usage of acetone-water/$NaHCO_3$ and acetone water/C_6H_5N [5] have also been reported. The application of coupling agents like *DCC* [6] and triethylamine in dichloromethane [7] or in tetrahydrofuran [3] is not desirable because of high cost and environment polluting solvents.

The environmentally friendly goal of synthesizing organic compounds without using solvents has been the subject of many investigations in recent years taking into account that drastic legistic restrictions on solvent pollution may require increased application of solventless reaction conditions in the future [8]. With the development of microwave ovens [9], reactions in dry media [10] have became easier to perform. Since microwaves are only adsorbed by the reactants on the

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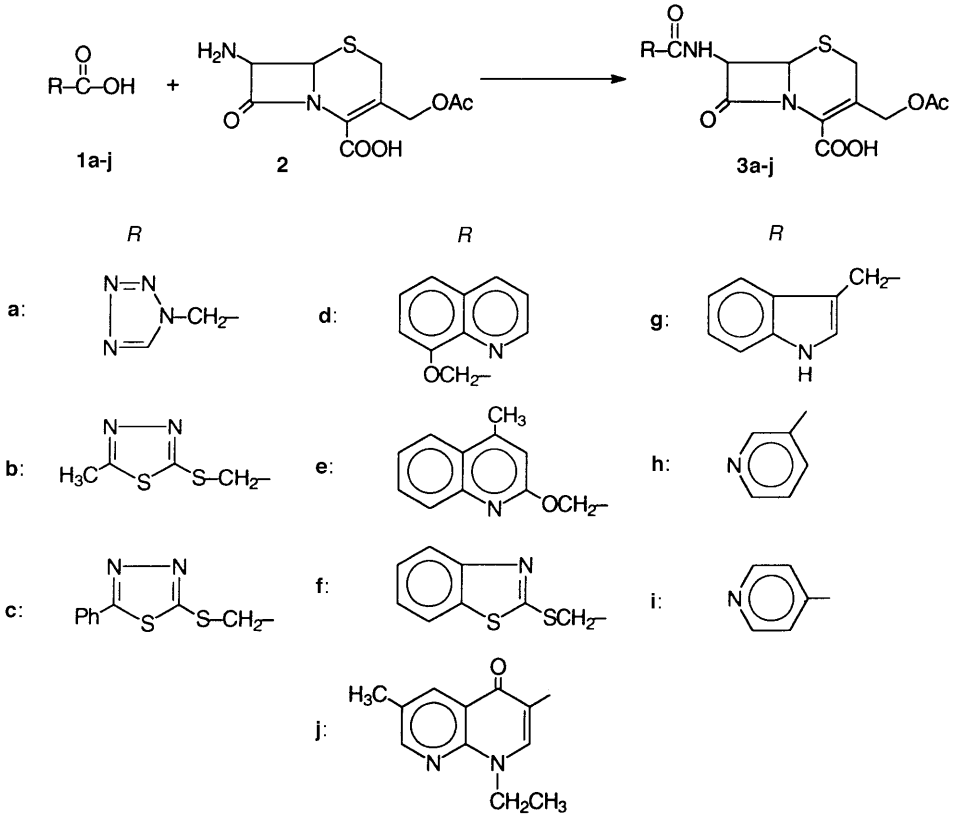
surface of common inorganic oxides such as silica and alumina [11], numerous reagents supported on solid surfaces [12] have been effectively utilized for conducting organic reactions under very safe and simple conditions using domestic microwave ovens. In general, microwave-assisted reactions may lend themselves to automation [13].

Though thousands of patents have been obtained for cephem derivatives, continuous efforts to synthesize new cephem derivatives [14] and to develop alternative synthetic methodologies are still in progress. In connection with the interest for new cephalosporins we describe a microwave-assisted synthesis using solid support [16–17] for the preparation of 7-substituted 8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-carboxylic acids. Their *in vitro* antibacterial profile against various bacteria strains is also discussed.

Results and Discussion

Syntheses

Compounds **3** were synthesized starting from the heterocyclic acids **1** and 7-ACA **2** (Scheme 1). **1a,g–j** are commercially available, **1b–f** were synthesized. Reaction of **2** with carboxylic acids **1a–j** by adsorption on basic alumina under microwave irradiation for 90–120 s afforded the 7-substituted cephalosporanic acid derivatives **3a–j**.



Scheme 1

Table 1. Reaction conditions and yields for compounds **3a–j**

	Conventional heating		Microwave irradiation	
	Yield/%	*t*/h	Yield/%	*t*/s
3a	65	3	87	90
3b	72	3	89	120
3c	63	4	87	90
3d	65	6	83	90
3e	63	3	85	120
3f	68	4	93	120
3g	67	2	82	90
3h	73	2	83	90
3i	72	3	92	120
3j	76	3	90	120

The structures attributed to the compounds are in accordance with the results of elemental analyses and with IR and ^{1}H NMR spectroscopic data.

The progress of the condensation of **1a–j** with **2** was monitored by the absence of starting material as detected by reverse phase HPLC. IR spectra of the condensed products **3a–j** displayed a band at 1700 cm^{-1} due to the side chain amide C=O group and a β-lactam C=O band at 1765 cm^{-1}. The shifting of the NH signal from 4.5 to 6.4 ppm ^{1}H NMR spectrum also confirmed the structure of the products. For the cephem skeleton, two doublets at 4.74 and 4.95 ppm with $J = 5.0$ Hz were assigned to the *cis*-hydrogens attached to C-6 and C-7.

Pharmacological results

E. herbicola, Corynbacterium, Z. mobilis, E. Coli, Enterobactor, K. aerogens, B. lichenformis, and *P. vulgaries* were used to determine the antibacterial activity by the cup diffusion method [18]. Cefotaxime and cephalothin were used as standards. All cephalosporin derivatives **3a–j** showed significant antibacterial activity against gram-positive as well as gram-negative organisms. Compounds **3a**,**d**,**e**,**i**,**j** were most potent and comparable to cephalothin acid (Table 2); only weak activity was observed with **3b**,**c**,**f**,**g**,**h**.

In conclusion, to the best of our knowledge this is the first report on the synthesis of cephalosporins using solid support under microwave irradiation. The salient feature of our approach is the combination of microwave technology with solvent-free conditions, thus achieving a modernization and simplification of the classical procedure, avoiding volatile and toxic organic solvents as well as corrosive mineral acids and bases. The results shown in Table 1 demonstrate the versatility of the process, bringing down the reaction time from hours to minutes with improved yield as compared to conventional heating [19].

Experimental

Melting points were determined by means of a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra (KBr pellets) were recorded on a Perkin-Elmer spectrophotometer model

Table 2. *In vitro* antibacterial activity[a] of compounds **3a–j**

	Erwinia herbicola 2491	*Corynbacterium rubrum* 2253	*Z. mobilis* 2873	*E. coli* K_{12}	*Enerobactor* 2340	*K. aerogens* 2281	*B. lichenformis* 2715	*P. vulgaries* 2027
3a	12.5	7.3	8.3	13.5	10.3	–[b]	–	9.9
3b	–	–	3.5	2.8	4.6	3.2	–	7.6
3c	3.2	1.5	3.6	–	3.5	6.3	7.5	6.4
3d	11.5	8.3	7.5	12.6	11.2	9.6	–	10.2
3e	11.7	8.6	8.3	11.9	10.3	8.7	6.5	10.8
3f	1.6	2.5	–	–	–	–	3.6	2.5
3g	–	3.5	–	–	6.5	5.2	4.3	5.7
3h	6.5	4.5	3.5	4.6	5.7	6.5	3.5	5.8
3i	11.5	8.3	9.2	12.8	11.2	11.3	10.5	9.7
3j	12.5	8.6	9.3	13.5	10.5	12.5	–	9.8
Cefotaxime acid	14.1	14.7	9.7	16.2	15.6	14.5	15.3	–
Cephalothin acid	12.2	7.9	8.7	15.4	13.5	–	–	10.3

[a] Zones of inhibition (mm); [b] no measurable activity

599. ^{1}H NMR spectra were recorded on a Perkin-Elmer R-32 (90 MHz) instrument using *TMS* as internal standard. Elemental analyses were performed by means of a Heraeus CHN rapid analyser; their results agreed satisfactorily with the calculated values. A Padmini Essentia microwave oven, Model Brownie, 2450 MHz, was used. For TLC analysis, silica gel coated Al plates (Merck) were employed.

General procedure for the synthesis of **1b–f**

To the solution of 5-methyl-1,3,4-thiadiazol-2-thiol, 5-phenyl-1,3,4-thiadiazol-2-thiol, 8-hydroxyquinoline, 2-hydroxy-4-methylquinoline, or benzothiazol-2-thiol (0.01 mol) in 5 cm^3 acetone, 18 g basic alumina [20] were added at room temperature. Ethyl bromo acetate (0.01 mol), adsorbed on basic alumina in a similar manner, was dried, properly mixed with the first batch, and irradiated in the microwave oven for 4–5 min. After completion of the reaction the mixture was cooled and extracted with acetone (4×10 cm^3). The acetone extracts were concentrated under reduced pressure to yield the corresponding acids.

5-Methyl-1,3,4-thiadiazol-2-yl-sulfanyl acetic acid (**1b**; $C_5H_6N_2O_2S_2$)

M.p.: 185–187 °C; ^{1}H NMR ($CDCl_3$, δ, 90 MHz): 2.65 (s, 3H, 5-CH_3), 4.52 (s, 2H, SCH_2) 10.1 (br, 1H, OH) ppm; IR (KBr): $\nu = 3150$ (OH), 1680 (C=O), 1520 (C=N) cm^{-1}.

5-Phenyl-1,3,4-thiadiazol-2-yl-sulfanyl acetic acid (**1c**; $C_{10}H_8N_2O_2S_2$)

M.p.: 170–172 °C, ^{1}H NMR ($CDCl_3$, δ, 90 MHz): 4.53 (s, 2H, SCH_2), 7.0–7.5 (m, 5H, ArH), 10.13 (br, 1H, OH) ppm; IR (KBr): $\nu = 3140$ (OH), 1690 (C=O), 1520 (C=N) cm^{-1}.

Quinolin-8-yloxy acetic acid (**1d**; $C_{11}H_9NO_3$)

M.p.: 153–155 °C; ^{1}H NMR ($CDCl_3$, δ, 90 MHz): 4.36 (s, 2H, OCH_2), 6.9–7.6 (m, 6H, ArH), 10.23 (br, 1H, OH) ppm; IR (KBr): $\nu = 3150$ (OH), 1690 (C=O) cm^{-1}.

4-Methylquinolin-2-yloxy acetic acid (**1e**; $C_{12}H_{11}NO_3$)

M.p.: 173–175 °C; ^{1}H NMR ($CDCl_3$, δ, 90 MHz): 2.18 (s, 3H, 4-CH_3), 4.49 (s, 2H, 2-OCH_2), 6.7 (s, 1H, 3-CH), 7.3 (t, 1H, 6-CH), 7.5 (t, 1H, 7-CH), 7.65 (d, 2H, 5- and 8-CH), 10.19 (br, 1H, OH) ppm; IR (KBr): $\nu = 3150$ (OH), 1675 (C=O) cm^{-1}.

Benzothiazol-2-yl-sulfanyl acetic acid (**1f**; $C_9H_7NO_2S_2$)

M.p.: 157–159 °C; ^{1}H NMR ($CDCl_3$, δ, 90 MHz): 4.42 (s, 2H, SCH_2), 7.1–7.5 (m, 4H, ArH), 10.23 (br, 1H, OH) ppm; IR (KBr): $\nu = 3130$ (OH), 1690 (C=O) cm^{-1}.

General procedure for the synthesis of **3a–j**

Basic alumina (18 g) was added to a solution of 2.72 g **2** (0.01 mol) dissolved in 3 cm^3 aqueous ammonia at room temperature. In another beaker, 10 g basic alumina were added to a solution of **1a–j** (0.01 mol) in acetone. The mixture was dried at room temperature, and the two reactants were thoroughly mixed using a mortar mixer and then placed in an alumina bath [21] inside the microwave oven. Upon completion of the reaction (90–120 s) as monitored by HPLC examination, the miture was cooled to room temperature, and the product was extracted into a mixture of water and acetic acid (1:4; 4×10 cm^3). Removal of the solvent under reduced pressure afforded the product which was purified by crystallization from a mixture of acetone/acetic acid.

7-((Tetrazol-1′-yl) acetylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid (**3a**; $C_{13}H_{14}N_6O_6S$)

M.p.: 215–217 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.7 (s, 3H, $COCH_3$), 4.2 (s, 2H, CH_2), 4.35 (s, 2H, OCH_2), 4.56 (s, 2H, NCH_2), 4.74 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.95 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.5 (br s, 1H, CONH), 8.9 (s, 1H, 5′-CH) ppm; IR (KBr): $\nu = 1790$ (C=O, β-lactam), 1680, 1640 (CONH and COOH), 1575 (C=N) cm^{-1}.

7-((5′-Methyl-1′,3′,4′-thiadiazol-2′-yl-sulfanyl)-acetylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**3b**; $C_{15}H_{16}N_4O_6S_3$)

M.p.: 206–208 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.7 (s, 3H, 5′-CH_3), 2.9 (s, 3H, $COCH_3$), 4.1 (s, 2H, CH_2), 4.3 (s, 2H, OCH_2), 4.56 (s, 2H, SCH_2), 4.7 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.9 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.3 (br s, 1H, CONH) ppm; IR (KBr): $\nu = 1785$ (C=O, β-lactam), 1675, 1642 (CONH and COOH), 1520 (C=N) cm^{-1}.

7-((5′-Phenyl-1′,3′,4′-thiadiazol-2′-yl-sulfanyl)-acetylamnio)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**3c**; $C_{20}H_{18}N_4O_6S_3$)

M.p.: 185–187 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.65 (s, 3H, $COCH_3$), 4.1 (s, 2H, CH_2), 4.42 (s, 2H, OCH_2), 4.53 (s, 2H, SCH_2), 4.7 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.93 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.2 (br s, 1H, CONH), 7.0–7.5 (m, 5H, 5′-ArH) ppm; IR (KBr): $\nu = 1788$ (C=O, β-lactam), 1680, 1640 (CONH and COOH), 1535 (C=N) cm^{-1}.

7-((Quinolin-5′-yl-oxy)-acetylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid (**3d**; $C_{21}H_{19}N_3O_7S$)

M.p.: 192–194 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.4 (s, 3H, $COCH_3$), 4.1 (s, 2H, CH_2), 4.3 (s, 2H, OCH_2) 4.46 (s, 2H, 8′-OCH_2), 4.56 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.73 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.1 (br s, 1H, CONH), 6.7–7.3 (m, 6H, ArH) ppm; IR (KBr): $\nu = 1780$ (C=O, β-lactam), 1670, 1630 (CONH and COOH) cm^{-1}.

7((4′-Methylquinolin-2′-yl-oxy)-acetylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**3e**; $C_{22}H_{21}N_3O_7S$)

M.p.: 207–209 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.2 (s, 3H, 4′-CH_3), 2.5 (s, 3H, $COCH_3$), 4.2 (s, 2H, OCH_2), 4.56 (s, 2H, 2′-OCH_2), 4.65 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.75 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.2 (br s, 1H, CONH), 6.82 (s, 1H, 3′-CH), 7.26 (t, 1H, 6′-CH), 7.59 (t, 1H, 7′-CH), 7.61 (d, 2H, 5′ and 8′-ArH) ppm; IR (KBr): $\nu = 1785$ (C=O, β-lactam), 1680, 1640 (CONH and COOH) cm^{-1}.

7-((Indol-3′-yl)-acetylamino)-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**3f**; $C_{20}H_{19}N_3O_6S$)

M.p.: 170-172 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.3 (s, 3H, $COCH_3$), 3.45 (s, 2H, 3′-CH_2), 4.2 (s, 2H, CH_2), 4.48 (s, 2H, OCH_2), 4.7 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.8 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.3 (br s, 1H, CONH), 7.3–7.6 (m, 4H, ArH), 9.0 (s, 1H, 2′-CH), 9.37 (br s, 1H, NH) ppm; IR (KBr): $\nu = 1790$ (C=O, β-lactam), 1680, 1635 (CONH and COOH) cm^{-1}.

7-((Benzothiazol-2′-yl-sulfanyl)-acetylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**3g**; $C_{20}H_{19}N_3O_6S_2$)

M.p.: 167–169 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.3 (s, 3H, $COCH_3$), 4.06 (s, 2H, CH_2), 4.28 (s, 2H, OCH_2), 4.45 (s, 2H, SCH_2), 4.6 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.9 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.3 (br s, 1H, CONH), 7.1–7.5 (m, 4H, ArH), 8.93 (s, 1H, 3′-CH) ppm; IR (KBr): $\nu = 1785$ (C=O, β-lactam), 1670, 1630 (CONH and COOH) cm^{-1}.

7-((Pyridin-3′-yl)-formylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**3h**; $C_{16}H_{15}N_3O_6S$)

M.p.: 188–190 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.6 (s, 3H, $COCH_3$), 4.2 (s, 2H, CH_2), 4.3 (s, 2H, OCH_2), 4.6 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.75 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.50 (br s, 1H CONH), 6.7–7.1 (m, 4H, ArH) ppm; IR (KBr): $\nu = 1795$ (C=O, β-lactam), 1680, 1645 (CONH and COOH) cm^{-1}.

7-((Pyridin-4′-yl)-formylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**3i**; $C_{16}H_{15}N_3O_6S$)

M.p.: 173–175 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 2.6 (s, 3H, $COCH_3$), 4.3 (s, 2H, CH_2), 4.5 (s, 2H, OCH_2), 4.7 (d, 1H, $J = 5.0$ Hz, 6-CH), 4.9 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.42 (br s, 1H, CONH), 6.8–7.3 (m, 4H, ArH) ppm; IR (KBr): $\nu = 1790$ (C=O, β-lactam), 1675, 1645 (CONH and COOH) cm^{-1}.

7-(N-Ethyl-6′-methyl-4′-oxo-naphthyridin-3′-yl)-formylamino)-3-acetyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]ot-2-ene-2-carboxylic acid (**3j**; $C_{22}H_{22}N_4O_7S$)

M.p.: 192–194 °C; ^{1}H NMR (*DMSO*-d_6, δ, 90 MHz): 16 (t, 3H, CH_2CH_3), 1.9 (t, 3H, CH_3), 2.5 (s, 3H, $COCH_3$), 3.3 (q, 2H, NCH_2), 4.4 (s, 2H, CH_2), 4.6 (s, 2H, OCH_2), 4.6 (d, 1H, $J = 5.0$ Hz, 6-CH),

4.8 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.3 (d, 1H, $J = 5.0$ Hz, 7-CH), 6.3 (br s, 1H, CONH), 8.5–8.9 (m, 2H, 5′-CH and 7′-CH), 9.6 (s, 1H, 2′-CH) ppm; IR (KBr): $\nu = 1785$ (C=O, β-lactam), 1670, 1640 (CONH and COOH) cm^{-1}.

Acknowledgements

P. Misra and *K. R. Bhushan* are thankful to the CSIR, New Delhi, for financial support.

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Received February 23, 2000. Accepted March 28, 2000