Reactions of Cephalosporin Sulfones 3. Synthesis of 2-Phenylhydrazonocephem-sulfones. A New Potential Entry to 2-Aminocephems [1]

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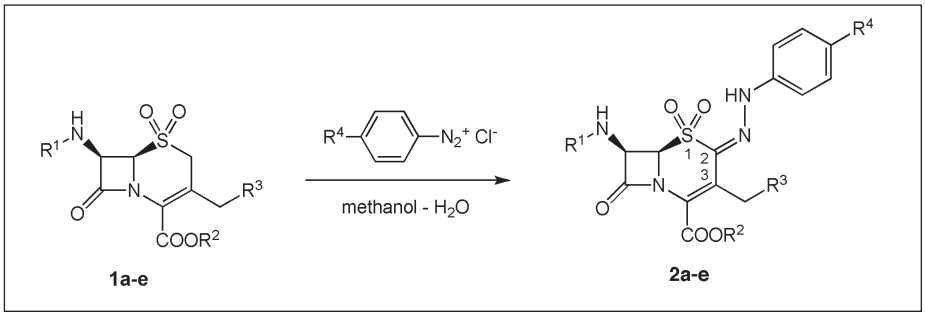

Reaction of cephem sulfones **1a-e** with aryldiazonium salts gives the 2-azo compounds which immediately rearrange into the corresponding 2-hydrazono derivatives **2a-e**.

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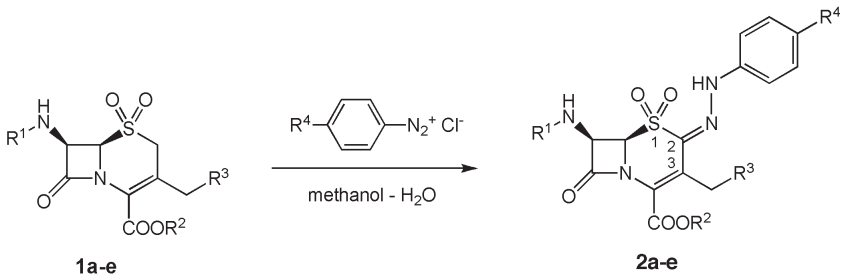
Introduction

Compared to the vast number of the cephalosporin C-3' derivatives, there are only a relatively small number of C-2 substituted cephalosporins. According to the SAR studies C-2 substituted cephalosporins never exhibited any markedly enhanced antibacterial activity compared to the parent compounds (similarly to the 2-substituted penicillins), therefore their chemical probing has always remained in the background. However, several 2-substituted penicillin sulfones exhibit excellent β-lactamase enzyme inhibitory properties, the most important is tazobactam (2ß-[(1,2,3-triazol-1-yl)methyl]-2α-methylpenam-3α-carboxylic acid 1,1-dioxid), which is commercially used in combinations with β-lactam antibiotics to provide broad-spectrum compositions against β-lactamase-producing microorganisms [2]. In addition, 2-substituted cephem sulfones were found to possess considerable human leucocyte elastase (HLE) inhibition properties [3]. Therefore it is of high importance to map the chemical and biological properties of 2-substituted cephalosporins as well.

During the search for appropriate reactions to prepare cephalosporin sulfones with new substituents at position 2, we tried to functionalize the C-2 carbon of the sulfones with aryl diazonium compounds. β-Diketones, sulfones and similar compounds carrying electron-withdrawing groups adjacent to hydrogens are known to form azo derivatives on coupling with aryldiazonium chlorides which sometimes underwent isomerization into the corresponding phenylhydrazones [4-6].

Results

In our experiments the cephalosporin sulfone **1a** was treated with phenyldiazonium chloride giving rise to a



	R^1	R^2	R^3	R^4
a	*t*-C_4H_9OCO-	-CH_3	-$OCOCH_3$	-H
b	$C_6H_5OCH_2CO$-	-CH_3	-H	-H
c	$C_6H_5OCH_2CO$-	-CH_3	-H	-Br
d	$C_6H_5OCH_2CO$-	-CH_3	-H	-NO_2
e	CH_3SO_2-	-*t*-C_4H_9	-H	-H

mixture of one major and several minor products. The major one was separated by chromatography to yield a golden yellow microcrystalline product. Its analytical data fully support the hydrazone structure **2a**: instead of an >CH-N=N-Ar type proton the NMR spectra revealed the presence of a new deuterium-exchangeable NH-proton. The characteristic ^{13}C NMR signal of the C-2 atom of the cephem sulfones at 51.85 ppm has been replaced by another one at 141.78 ppm, characteristic to the newly formed hydrazon carbon. In addition to the spectroscopic and analytical evidences, we intended to confirm its structure further with X-ray structural analysis, but no acceptable crystals of **2a** could be obtained. Therefore the 4-bromo- and 4-nitrophenyl derivatives **2c** and **2d** were also prepared. Unfortunately, their silky needle crystals were inappropriate for X-ray analyses.

Altough the hydrazone moiety would exist in *E* or *Z* conformation, the NMR data of the isolated compounds clearly support the presence of the *Z* isomers:

a) The NH proton of the 2-hydrazono group displays a strong downfield shift (~11.2 ppm in deuteriochloroform) and it is extremely slowly exchangeable with deuterium oxide. This indicates a strong hydrogen bond between the hydrazone NH proton and one of the oxygen atoms of the sulfone group.

b) A $\{^1H\}$-^1H NOE experiment was performed involving the protons of the hydrazone moiety and those in its immediate proximity. Additionally, we carried out AM1 QM calculations to obtain reasonable geometries of the *E* and *Z* isomers. The NMR and NOE data are in full agreement with the *Z* isomer. Finally, QM modelling shows that in the case of the *E*-isomer a close contact between the hydrazone phenyl group and the C-3 methyl group would result in a considerably more strained structure with a higher energy of about 10 kcal/mol.

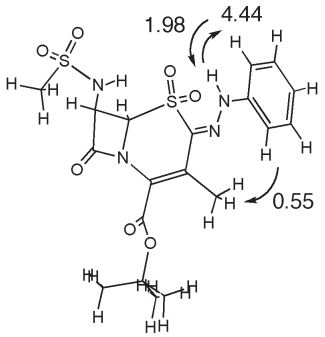


Figure 1. AM1 refined structure of the *E* isomer of **2e** and the observed $\{^1H\}$-^1H NOE interactions.

In conclusion, an efficient synthetic route has been found for the preparation of 2-hydrazonocephem sulfones which may serve as starting materials for new 2-*N*-substituted cephalosporins. According to preliminary experiments mild reduction of the hydrazones lead to the mixtures of the corresponding phenylhydrazine componds and rearranged seven-membered heterocycles. This will be subject of a forthcoming paper.

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EXPRIMENTAL

The ^1H nmr spectra were recorded with BRUKER-200 spectrometer and shifts are given in ppm relative to internal tetramethylsilane. The ir spectra were measured by FTIR (Perkin-Elmer PC-11). Melting points were measured with a Koffler-type apparatus and are not corrected. Thin layer chromatography was carried out on aluminum foil coated with silica gel 60 F_{254} (Merck), column chromatography on silica gel 60 (Merck).

Preparation of the starting cephalosporine sulfones.

Methyl 7β-(Phenoxyacetylamino)-3-deacetoxycephalosporanate 1,1-dioxide (**1b**)

a) 7β-(Phenoxyacetylamino)-3-deacetoxycephalosporanic acid: 7-Amino-3-deacetoxycephalosporanic acid (5.35 g, 25 mmole) and 5 g of sodium bicarbonate were dissolved in a mixture of 50 mL of acetone and 100 mL of water. Phenoxyacetylchloride (4.32 g, 3.5 mL, 25 mmole, dissolved in 15 mL of dry acetone) was added dropwise while stirring and maintaining the temperature between 0-5 °C. The pH of the reaction mixture was checked from time to time and a small amount of solid sodium bicarbonate was added when necessary to maintain it slightly above 7.0. After 4 hours the acetone was removed *in vacuo*, 100 mL of ethyl acetate was added to the remaining solution and its pH was lowered to 2 with 40% aqueous phosphoric acid. The organic layer was separated, the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were dried and used immediately for the next step.

b) Methyl 7β-(Phenoxyacetylamino)-3-deacetoxycephalosporanate: To the above ethyl acetate solution an etheral solution of diazomethane was added slowly in several portions under an efficient hood. The reaction was followed by thin layer chromatography (toluene-ethyl acetate-acetic acid 7:3:1). When no starting material could be observed, the excess diazomethane was decomposed by addition of a few drops of acetic acid. The mixture was washed with a 10% aqueous solution of sodium bicarbonate, dried over magnesium sulfate nad evaporated. The residue was recrystallized from methanol – ether providing 5.0 g of white product (83% overall), mp 135-136 °C. It was used without further purification for the next oxidation step.

c) Methyl 7β-(Phenoxyacetylamino)-3-deacetoxycephalosporanate 1,1-dioxide: The above methyl ester (5.0 g) was dissolved in 60 mL of dichloromethane and 8.96 g of 3-chloro-peroxybenzoic acid (~55%, 2 eq.) was added in a few portions. The mixture was stirred for 4 hours at room temperature and another portion of 3-chloro-peroxybenzoic acid (2.2 g) was added to complete the reaction (thin layer chromatography, toluene – ethyl acetate 1:1). Next day the reaction mixture was diluted with 50 mL of dichloromethane to clear the solution and it was washed by 10% aqueous sodium bicarbonate solution and water. Evaporation and recrystallization from methanol – ether gave rise to 4.38 g (81%) of white product, mp 188-190 °C; ir (KBr): ν 1788, 1712, 1696, 1224, 1230, 1130 cm^{-1}; ^1H nmr (200

MHz, deuteriodimethylsulfoxide): δ 1.99 (3H, s, 3-CH_3), 3.78 (3H, s, $COOCH_3$), 4.23, 4.37 (2H, ABq, J = 18.20 Hz, 2-CH_2), 5.33 (H, d, J = 4.68 Hz, 6-H), 6.04 (H, dd, J = 4.68, 9.39 Hz, 7-H), 6.86-7.00 (5H, m, aromatic), 8.60 (H, d, J = 9.39 Hz, NH); ^{13}C nmr (500 MHz, deuteriodimethylsulfoxide): δ 19.40, 52.54, 55.36, 57.61, 66.81, 67.10, 114.70, 121.26, 121.99, 129.48, 130.43, 156.83, 161.18, 163.10, 168.27.

Anal. Calcd for $C_{17}H_{18}N_2O_7S$ (MW 394.08) C, 51.77; H, 4.60; N, 7.10. Found: C, 51.99; H, 4.58; N: 7.23.

tert-Butyl 7β-(Methylsulfonylamino)-3-deacetoxycephalosporanate 1,1-dioxide (**1e**)

a) *tert*-Butyl 7β-Amino-3-deacetoxy-cephalosporanate was prepared from 7-amino-3-deacetoxycephalosporanic acid and *tert*-butylacetate by BF_3·Et_2O catalysis according to the method of Grigan *et al.* [7].

b) The above ester was mesylated to *tert*-butyl 7β-(methylsulfonylamino)-3-deacetoxy-cephalosporanate using methanesulfonyl chloride [8], mp 148-149 °C (ethyl acetate – hexane); ^{1}H nmr (200 MHz, deuteriochloroform): δ 1.52 (9H, s, 4-$COOC(CH_3)_3$), 2.11 (3H, s, 3-CH_3), 3.14 (3H, s, 7-CH_3SO_2), 3.24, 3.50 (2H, ABq, J = 19.5 Hz, 2-CH_2), 4.99 (H, d, J = 4.8 Hz, 6-H), 5.25 (H, dd, J = 4.8, 10.4 Hz, 7-H), 5.61 (H, d, J = 10.4 Hz, 7-NH).

c) The above *tert*-butyl 7β-(methylsulfonylamino)-3-deacetoxycephalosporanate (1.55 g) was dissolved in 30 ml of ethyl acetate and 3ml of 30% hydrogen peroxide solution was added followed by 0.1 g of sodium tungstate. The reaction mixture was stirred at 50 °C for 24 hours. The product partly crystallized out from the solution, it was filtered off and rinsed thoroughly with water. The organic solution was washed with water, dried over magnesium sulfate and evaporated to yield another crop of the product. The combined solid products were recrystallized from isopropyl alcohol – acetone yielding 1.26 g of white product (77%), mp 197-201 °C; ^{1}H nmr (200 MHz, deuteriochloroform): δ 1.53 (9H, s, 4-$COOC(CH_3)_3$), 2.13 (3H, s, 3-CH_3), 3.15 (3H, s, 7-CH_3SO_2), 3.63, 3.88 (2H, ABq, J = 18.0 Hz, 2-CH_2), 4.83 (H, d, J = 4.7 Hz, 6-H), 5.47 (H, dd, J = 4.7, 11.6 Hz, 7-H), 5.95 (H, d, J = 11.6 Hz, 7-NH) ; ^{13}C nmr (500 MHz, deuteriochloroform): δ 18.96, 27.40, 41.54, 55.05, 61.54, 67.15, 83.05, 122.50, 127.62, 159.40, 162.34.

Standard diazotation procedure.

Aniline (1.9 g, 0.02 mole, or the equivalent amount of 4-chloro- or 4-nitroaniline) was dissolved in 10 ml of 1:1 mixture of cc. HCl – water. The mixture was cooled to 0-5 °C and a solution of of 1.4 g $NaNO_2$ in 7 mL of water was added dropwise while the temperature was maintained at this temperature. This solution was used for the subsequent coupling reactions.

Methyl 7-*tert*-Butoxycarbonylamino-2-phenylhydrazono-cephalosporanate 1,1-dioxide (Methyl (6*R*,7*R*)-3-[(Acetyloxy)-methyl]-7-[(*tert*-butoxycarbonyl)amino]-8-oxo-4-(phenyl-hydrazono)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide) (**2a**).

To an ice-cold solution of 200 mg of **1a** [9] in 20 ml of methanol a hydrochloric acid solution of diazoted aniline was added in several portions (prepared from 1.9 ml of aniline as described above) until no starting material could be detected by thin layer chromatography. After 1 hour in the cold the reaction mixture was diluted with 30 mL of water, extracted twice with dichloromethane (10 mL). The organic phase was washed with water, 10% sodium bicarbonate solution and water. After evaporation the residue was chromatographed (silica gel, toluene–EtOAc 3:1) yielding 62 mg of bright yellow microcrystalline product (**2a**), mp 166-168 °C (from isopropyl alcohol); ir (KBr): ν 1806, 1730, 1532, 1510, 1240 cm^{-1}; ms: *m/z* (70eV) 522 (M^+), 365 (M-157); ^{1}H nmr (200 MHz, deuteriochloroform) δ 1.93 (9H, s, $C(CH_3)_3$), 2.50 (3H, s, -$COCH_3$), 3.84 (3H, s, -OCH_3), 5.44, 5.80 (2H, ABq, J = 11.8 Hz, 3'-CH_2), 6.25 (H, d, J = 4.5 Hz, H-6), 6.44 (H, dd, J = 4.5 Hz, 8.8 Hz, H-7), 7.55-7.9 (5H, m, Ph), 8.26 (H, d, J = 8.8 Hz, 7-NH), 11.45 (H, s, NHPh); ^{13}C nmr (500 MHz, deuteriodimethylsulfoxide): δ 21.26, 28.59, 53.74, 58.12, 63.14, 70.68, 82.30, 115.14, 123.12, 123.25, 125.48, 126.58, 130.18, 141.78, 154.48, 161.59, 163.84, 171.05.

Anal. Calcd for $C_{22}H_{26}N_4O_9S$ (MW 522.53): C, 50.57; H, 5.02; N, 10.72; S, 6.14. Found: C, 50.28; H, 5.09; N, 10.67; S, 6.59.

2b, c and **d** were similarly prepared as **2a**:

Methyl 7β-[(Phenoxyacetyl)amino-2-(phenylhydrazono)-3-deacetoxycephalosporanate 1,1-dioxide (Methyl (6*R*,7*R*)-3-Methyl-8-oxo-7-[(phenoxyacetyl)amino]-4-(phenylhydrazono)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide) (**2b**):

Mp 160-162 °C; ir (KBr): ν 1802, 1704, 1530, 1488,1264, 1242, 1170 cm^{-1}; ^{1}H nmr (200 MHz, deuteriodimethylsulfoxide): δ 2.28 (3H, s, 3-CH_3), 3.82 (3H. s, $COOCH_3$), 4.62, 4.72 (2H, ABq, 2H, J = 15.3, O*C*H_2CONH), 5.82 (H, d, J = 4.6 Hz, 6-H), 6.20 (H, dd, J = 4.6, 6.2 Hz, 7-H), 6.9-7.6 (10H, m, 10H, aromatic), 8.87 (H, d, J = 6.2 Hz, 7-NH), 11.52 (H, s, 2-NH-Ar); ^{13}C nmr (500 MHz, deuteriochloroform): δ 14.37, 53.28, 59.29, 67.37, 70.61, 115.23, 115.31, 119.72, 122.80, 125.13, 125.29, 130.08, 130.20, 132.08, 142.00, 157.28, 162.38, 162.52, 168.91.

Anal. Calcd for $C_{23}H_{22}N_4O_7S$ (MW 498.52) C, 55.42; H, 4.45; N, 11.24. Found: C, 55.02; H, 4.35; N: 11.29.

Methyl 7β-[(Phenoxyacetyl)amino-2-[(4-bromophenyl)hydrazono]-3-deacetoxycephalosporanate 1,1-dioxide (Methyl (6*R*,7*R*)-3-Methyl-8-oxo-7-[(phenoxyacetyl)amino]-4-[(4-bromophenyl)phenylhydrazono]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide) (**2c**):

Mp 193-195 °C; ^{1}H nmr (200 MHz, deuteriodimethylsulfoxide): δ 2.26 (3H, s, 3-CH_3), 3.82 (3H, s, $COOCH_3$), 4.63, 4.74 (2H, ABq, J = 14.9 Hz, O*C*H_2CONH), 5.82 (H, d, J = 4.7 Hz, 6-H), 6.23 (H, dd, J = 4.7, 6.3 Hz, 7-H), 6.9-7.9 (9H, m, aromatic), 8.84 (H, d, J = 6.3 Hz, 7-NH), 11.55 (2-NH-Ar); ^{13}C nmr (500 MHz, deuteriodimethylsulfoxide): δ 14.86, 53.62, 59.63, 67.01, 70.49, 115.47, 116.58, 118.09, 120.29, 122.14, 127.69, 130.40, 132.98, 139.60, 142.88, 158.33, 162.82, 163.26, 169.02.

Anal. Calcd for $C_{23}H_{21}BrN_4O_7S$ (MW 577.41) C, 47.84; H, 3.67; N, 9.70; Br: 13.84. Found: C, 47.64; H, 3.77; N: 9.66; Br, 13.51.

Methyl 7β-[(Phenoxyacetyl)amino-2-[(4-nitrophenyl)hydrazono]-3-deacetoxycephalosporanate 1,1-dioxide (Methyl (6*R*,7*R*)-3-Methyl-8-oxo-7-[(phenoxyacetyl)amino]- 4-[(4-nitrophenyl)phenylhydrazono]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide) (**2d**):

Mp 226-227 °C; ^{1}H nmr (200 MHz, deuteriodimethylsulfoxide): δ 2.27 (3H, s, 3-CH_3), 3.85 (3H, s, $COOCH_3$), 4.63, 4.74 (2H, ABq, J = 15.4, O*C*H_2CONH), 5.90 (H, d, H, J = 5.0 Hz, 6-

H), 6.25 (H, dd, J = 5.0, 8.0 Hz, 7-H), 6.9, 7.3 (5H, m, 7-aromatic), 7.62, 8.13 (4H, m, 2-aromatic), 8.96 (H, d, J = 8.0 Hz, 7-NH), 11.87 (2-NH-Ar); ^{13}C nmr (500 MHz, deuteriodimethylsulfoxide): δ 14.92, 53.79, 59.75, 66.99, 70.65, 115.46, 116.07, 121.98, 122.23, 126.41, 129.41, 130.46, 131.06, 143.43, 148.91, 158.36, 162.67, 163.09, 169.07.

Anal. Calcd for $C_{23}H_{21}N_5O_9S$ (MW 543.52) C, 50.83; H, 3.89; N, 12.89. Found: C, 51.03; H, 3.81; N: 12.55.

tert-Butyl 7β-[(Methylsulfonyl)amino]-2-phenylhydrazono-3-deacetoxycephalosporanate 1,1-dioxide (*tert*-Butyl (6*R*,7*R*)-3-Methyl-7-[(methylsulfonyl)amino]-8-oxo-4-(phenylhydrazono)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide) (**2e**):

This compound was preparated similarly to **1a**, but a mixture of methanol – pyridine (1 : 2) was used as solvent. After the solvent was evaporated off *in vacuo*, dichloromethane was added and worked up as above. Mp 209-211 °C; ^{1}H nmr (200 MHz, deuteriochloroform): δ 1.55 (9H, s, 4-COOC(CH_3)$_3$), 2.36 (3H, s, 3-CH_3), 3.18 (3H, s, 7-CH_3SO_2), 55.11 (H, d, J = 5.3 Hz, 6-H), 5.56 (H, dd, J = 5.3, 11.4 Hz, 7-H), 6.03 (H, d, J = 11.4 Hz, 7-NH), 7.05-7.4 (5H, m, aromatic), 11.15 (2-NH-Ar); ^{13}C nmr (500 MHz, deuteriodimethylsulfoxide): δ 14.68, 28.37, 41.79, 63.44, 70.84, 83.75, 115.86, 121.82, 124.64, 127.31, 128.66, 130.24, 143.49, 161.32, 162.75.

Anal. Calcd for $C_{19}H_{24}N_4O_7S_2$ (MW 484.55) C, 47.10; H, 4.99 N, 11.56. Found: C, 47.22; H, 4.85; N: 11.51.

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