*Journal of Organometallic Chemistry*, 107 (1976) 351—357


# A NEW CLASS OF SEMI-SYNTHETIC ANTIBIOTICS: FERROCENYL-PENICILLINS AND -CEPHALOSPORINS*

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(Received September 23rd, 1975)

## Summary

A series of ferrocenyl carboxylic acids has been prepared and condensed, via their acid chlorides or in the presence of *N,N'*-dicyclohexylcarbodiimide, with both 6-aminopenicillanic acid and 7-aminocephalosporanic acid. Almost all of the ferrocenyl-penicillins and -cephalosporins exhibited antibiotic activity, some being highly active, while others proved to be potent β-lactamase inhibitors.

## Introduction

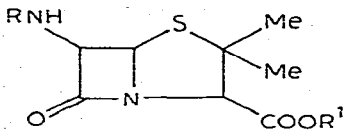
The semi-synthetic penicillins and cephalosporins are an important group of antibiotics. They are usually prepared by condensation of the amino group of 6-aminopenicillanic acid (Ia; $R^1$ = H), or 7-aminocephalosporanic acid (IIa; $R^1$ = H) with an aromatic acid. The introduction of substituents into these molecules modifies their microbiological spectrum, acid stability, metabolic efficiency, degree of binding to serum proteins and, perhaps most important, resistance to β-lactamases.

Since the introduction of penicillins and cephalosporins into clinical practice, a continual problem has been the emergence of drug-resistant strains of bacteria [2]. In many cases this resistance arises from the production of β-lactamases, enzymes which degrade these antibiotics. Synthesis of new penicillins and cephalosproins is important to keep pace with the appearance of these resistant bacterial strains.

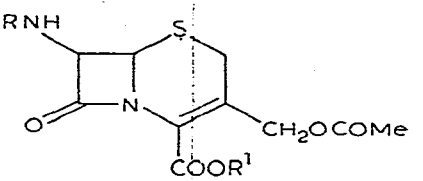
The most widely used method for the introduction of side-chain substituents into 6-aminopenicillanic acid (Ia, $R^1$ = H) and 7-aminocephalosporanic acid (IIa, $R^1$ = H) is by condensation with the appropriate acid chloride in the presence of a tertiary amine [3]. Other methods of introducing substituents into the side-chain include, condensation with an acid anhydride [3] or activated ester [4] and direct coupling of an acid in the presence of *N,N'*-dicyclohexyl-

* Some of these results have been presented in a preliminary form [1].

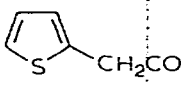
carbodiimide [5]. In all cases the resulting penicillin, or cephalosporin is isolated as an alkali metal salt, either by freeze-drying or by precipitation.



(I)

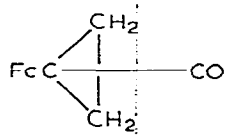


(II)

(a) R = H; (b) R = $PhCH_2CO$; (c) R =  ; (d) R = FcCO;

(e) R = $FcCH_2CO$; (f) R = FcCH(Me)CO; (g) R = $FcC(Me)_2CO$;

(Fc = $C_{10}H_9Fe$)

(h) R = 

Many semi-synthetic penicillins and cephalosporins possess a side-chain that incorporates an aromatic or heteroaromatic group, for example, benzylpenicillin (Ib, $R^1$ = K) and cephalothin (IIc, $R^1$ = Na) are in current clinical use. In this report we describe the preparation of ferrocenyl-penicillins and -cephalosporins derivatives. With the exception of a number of organophosphorus derivatives of penicillin [6] there are apparently no previous reports of molecules of this type, that is, having organometallic substituents in the side-chain.

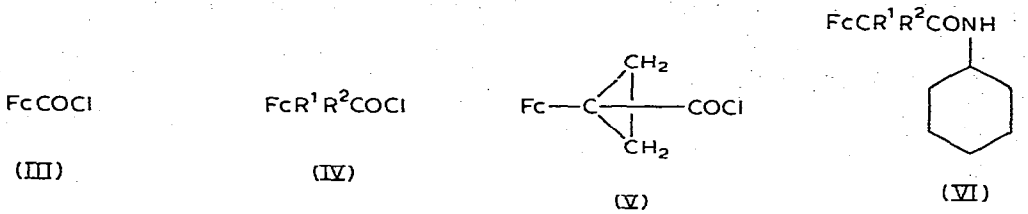
## Results and discussion

The aromatic character, stability, low toxicity [7] and ease of substitution of ferrocene makes it ideal for use in drug design. Also, the sandwich structure of ferrocene renders it completely different from the conventional aromatic molecules usually attached to the penicillin and cephalosporin nuclei. A metal atom is placed in close proximity to the β-lactam ring and the second cyclopentadienyl ring modifies the molecule in the third dimension without disturbing the molecular profile necessary for antibiotic activity.

Ferrocene carboxylic acid [8], ferrocenyl acetic acid [9] and 2-ferrocenylpropionic acid [9,10] were prepared via well established routes. 2-Ferrocenyl-2-methylpropionic acid and ferrocenyl-cyclopropane carboxylic acid were prepared by alkaline hydrolysis of the corresponding cyanides [11]. Treatment of these acids with phosphorus(III) chloride [12] afforded the acid chlorides III, IVa, IVb, IVc and V respectively. The chlorides IVa, IVb and IVc were characterised by condensation with cyclohexylamine to give the amides VIa, VIb and VIc respectively.

Ferrocenylacetyl chloride (IVa) was condensed with a molar equivalent

of 6-aminopenicillanic acid (Ia, $R^1$ = H) in the presence of two molar equivalents of triethylamine. Acidification of an aqueous solution of the triethyl-




(III) (IV) (V) (VI)

(a) $R^1 = R^2 = H$ ; (b) $R^1 = H, R^2 = Me$; (c) $R^1 = R^2 = Me$

amine salt afforded 6-(2-ferrocenylacetamido)-penicillanic acid which was isolated as its sodium salt (Ie, $R^1$ = Na) by the addition of sodium 2-ethylhexanoate [13]. Thin layer chromatography showed the compound (Ie, $R^1$ = Na) to be pure. The infrared spectrum showed a broad absorbance at 3455 $cm^{-1}$ (OH), which was attributed to water of crystallization [14], and carbonyl absorbances characteristic of a penicillin nucleus. Infrared spectroscopy is a useful physical method for the identification of penicillins and cephalosporins. The β-lactam carbonyl has a characteristic stretching frequency in the range 1790—1750 $cm^{-1}$ while the carbonyl stretching band for the secondary amide group is located in the region 1690—1650 $cm^{-1}$ [15]. Carbonyl stretching frequencies for the ferrocenyl-penicillin and -cephalosporin derivatives are given in Table 1.

The ferrocenyl-penicillins Id, If, Ig and Ih ($R^1$ = Na) and ferrocenyl-cephalo-

TABLE 1

CARBONYL STRETCHING FREQUENCIES ($cm^{-1}$) FOR SOME FERROCENYL-PENICILLIN AND -CEPHALOSPORIN DERIVATIVES

| Compound | $R^1$ = | β-lactam carbonyl | Secondary amide carbonyl | Ester carbonyl |
|---|---|---|---|---|
| Id | Na [a] | 1760 | 1660 | — |
| Id | $C_{10}H_{15}^{+}NH_3$ | 1765 | 1640 | — |
| Ie | Na | 1760 | 1675 | — |
| Ie | $PhCH_2^{+}NH_3$ | 1765 | 1655 | — |
| If | Na | 1750 | 1660 | — |
| If | $PhCH_2^{+}NH_3$ | 1760 | 1660 | — |
| If | $C_6H_{11}^{+}NH_3$ | 1775 | 1660 | — |
| Ig | Na | 1755 | 1670 | — |
| Ig | $PhCH_2^{+}NH_3$ | 1760 | 1665 | — |
| Ig | $C_6H_{11}^{+}NH_3$ | 1775 | 1670 | — |
| Ih | Na [a] | 1740 | 1680 | — |
| Ih | $C_6H_{11}^{+}NH_3$ | 1765 | 1670 | — |
| IIe | Na | 1750 | 1655 | 1735 |
| IIe | $C_{10}H_{15}^{+}NH_3$ | 1775 | 1665 | 1735 |
| IIf | Na [a] | 1750 | 1665 | 1730 |
| IIf | $C_{10}H_{15}^{+}NH_3$ | 1765 | 1665 | 1735 |
| IIg | Na [a] | 1755 | 1665 | 1735 |
| IIg | $C_{10}H_{15}^{+}NH_3$ | 1755 | 1675 | 1735 |

[a] Characterised as amine salts (Table 2).

sporins IId, IIe, IIf and IIg ($R^1$ = Na) were prepared in a similar manner. Also, direct condensation of ferrocenylacetic acid with 6-aminopenicillanic acid Ia ($R^1$ = H) in the presence of *N,N'*-dicyclohexylcarbodiimide afforded the ferrocenyl-penicillin Ie ($R^1$ = Na) which was identical to that obtained by the acid chloride route. Each ferrocenyl-penicillin and -cephalosporin was characterised by the preparation of at least one amine salt. These amines included benzylamine, cyclohexylamine and adamantanamine. The latter amine was found to be particularly useful in the preparation of ferrocenyl-cephalosporin salts since cephalosporins in general are more susceptible to hydrolysis under alkaline conditions than penicillins, and adamantanamine gives solutions of a lower pH than benzylamine or cyclohexylamine. Adamantanamine also proved useful in the characterisation of labile ferrocenyl-penicillins when other amine salts could not be isolated as crystalline solids.

The ferrocenyl-penicillins and -cephalosporins were tested in vitro for antibiotic activity against a number of different bacteria, including some penicillinase producing strains. Benzyl penicillin or methicillin was used as a control for the penicillin derivatives, while cephalothin was used for the cephalosporins. The method of testing involved the finding of the lowest concentration, in μg/ml, of penicillin, or cephalosporin, required to inhibit growth of each bacterium. This is termed the minimum inhibitory concentration (M.I.C.). The tests showed that compounds Id ($R^1$ = Na) and IId ($R^1$ = Na) were inactive. This may be due to the close proximity of the metal atom to the β-lactam ring, or to steric hindrance from the bulky ferrocene group. In contrast the ferrocenyl-penicillin Ie ($R^1$ = Na) exhibited high antibiotic activity, comparable to that of benzyl penicillin, but the activity of the other penicillin derivaitves If, Ig and Ih ($R^1$ =

TABLE 2

ANALYTICAL DATA FOR SOME FERROCENYL-PENICILLIN AND -CEPHALOSPORIN DERIVATIVES

| Compound | $R^1$ = | Yield (%) | M.p. (°C)(dec.) | Empirical formula | Found (calcd.) (%) | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| Id | $C_{10}H_{15}^{+}NH_3$ | 50 | 169—171 | $C_{29}H_{37}FeN_3O_4S$ | 59.52 (60.10) | 6.72 (6.43) | 7.06 (7.24) |
| If | Na | 50 | 197—200 | $C_{21}H_{23}FeN_2NaO_4S \cdot H_2O$ | 50.86 (50.82) | 5.00 (5.08) | 5.69 (5.64) |
| If | $PhCH_2^{+}NH_3$ | 64 | 97—100 | $C_{28}H_{33}FeN_3O_4S \cdot \frac{1}{2}H_2O$ | 58.70 (58.74) | 6.14 (5.98) | — — |
| If | $C_6H_{11}^{+}NH_3$ | 67 | 135—138 | $C_{27}H_{37}FeN_3O_4S$ | 58.29 (58.37) | 6.88 (6.72) | 7.54 (7.57) |
| Ig | Na | 82 | 202—205 | $C_{22}H_{25}FeNaN_2O_4S \cdot 2H_2O$ | 49.81 (50.01) | 5.70 (5.53) | 5.28 (5.30) |
| Ig | $PhCH_2^{+}NH_3$ | 59 | 103—105 | $C_{29}H_{35}FeN_3O_4S \cdot H_2O$ | 58.74 (58.48) | 6.36 (6.26) | — — |
| Ig | $C_6H_{11}^{+}NH_3$ | 61 | 134—136 | $C_{28}H_{39}FeN_3O_4S$ | 58.98 (59.05) | 6.97 (6.91) | 7.56 (7.38) |
| Ih | $C_6H_{11}^{+}NH_3$ | 61 | 149—152 | $C_{28}H_{37}FeN_3O_4S$ | 58.98 (59.25) | 6.62 (6.58) | 7.32 (7.41) |
| IIf | $C_{10}H_{15}^{+}NH_3$ | 57 | 153—156 | $C_{33}H_{41}FeN_3O_6S$ | 59.19 (59.70) | 6.50 (6.23) | 6.22 (6.34) |
| IIg | $C_{10}H_{15}^{+}NH_3$ | 54 | 164—166 | $C_{34}H_{43}FeN_3O_6S$ | 59.89 (60.27) | 6.60 (6.40) | 6.06 (6.21) |

Na) decreased with increasing substitution of the α-ferrocenyl carbon atom. The ferrocenyl-cephalosporins also showed antibiotic activity but they were less active than the cephalothin control. The ferrocenyl-penicillins Ie, If and Ig ($R^1$ = Na) and, to a lesser extent, the cephalosporins IIe, IIf and IIg ($R^1$ = Na) all behaved as β-lactamase inhibitors. The α-dimethyl compound Ig ($R^1$ = Na) proved to be the best inhibitor since the degree of inhibition increased with increasing substitution of the α-ferrocenyl carbon atom.

## Experimental

*2-Ferrocenyl-2-methylpropionic acid*

A suspension of 2-ferrocenyl-2-methylpropanonitrile, (9.68 g, 0.038 mol) in ethanol (200 ml) was added to a solution of potassium hydroxide (25 g) in water (200 ml) and the mixture heated under reflux. After the evolution of ammonia had ceased (48 h), the ethanol was removed in vacuo. The residual suspension was dissolved in water (200 ml), extracted with ether and filtered. Acidification of the alkaline solution with 90% orthophosphoric acid afforded a yellow solid. The product, 2-ferrocenyl-2-methylpropionic acid, was collected by filtration (7.84 g, 76%) and crystallized from ethanol/water as bright yellow needles, m.p. 163—165°C, (Found: C, 61.53; H, 5.85; mol. wt., 272 by mass spectrometry. $C_{14}H_{16}FeO_2$ calcd.: C, 61.82; H, 5.93%; mol. wt., 272.13). PMR (60 MHz) (δ, $CD_3OD$): 4.14 (9H, s, ferrocenyl), 1.45 (6H, s, methyl).

*2-Ferrocenyl-2-methylpropanoyl chloride (IVc)*

2-Ferrocenyl-2-methylpropionic acid (7.55 g, 0.028 mol) and pyridine (15 drops) was dissolved in dry benzene (500 ml) and phosphorus(III) chloride (25 ml) was added dropwise. The mixture was stirred for 3 h at 60—70°C. The benzene solution was then decanted off and evaporated under vacuum. The red-brown viscous residue was dissolved in benzene and the benzene evaporated. This procedure was repeated. 2-Ferrocenyl-2-methylpropanoyl chloride (6.91 g, 85%) was obtained as a red-brown solid which was recrystallised from light petroleum (b.p. 40—60°C) immediately before use. 2-Ferrocenyl-2-methylpropanoyl chloride was characterised by condensation with cyclohexylamine to give *N*-cyclohexyl-2-ferrocenyl-2-methylpropanamide (VIc) which was recrystallised from ether/light petroleum (b.p. 40—60°C) as deep yellow needles, m.p. 92—94°C, (Found: C, 68.08; H, 7.70; mol. wt., 353 by mass spectrometry. $C_{20}H_{27}FeNO$ calcd.: C, 67.98; H, 7.71%; mol. wt. 353.30). PMR (60 MHz) (δ, $CCl_4$): 5.15 (1H, broad s, N—H), 4.08 (9H, s, ferrocenyl), 1.90—0.80 (17H, multiplet with singlet at 1.43, methyl and cyclohexyl).

*Acid chlorides IVa and IVb and their condensation with cyclohexylamine*

The acid chlorides IVa and IVb were prepared as previously described and condensation of ferrocenylacetyl chloride (IVa) with cyclohexylamine afforded *N*-cyclohexyl-ferrocenylacetamide (VIa) which crystallised from ether/light petroleum (b.p. 40—60°C) as bright yellow needles, m.p. 139—141°C (Found: C, 66.61; H, 7.15; mol. wt., 325 by mass spectrometry. $C_{18}H_{23}FeNO$ calcd.: C, 66.47; H, 7.13%; mol. wt. 325.24). Similarly 2-ferrocenylpropanoyl chloride (IVb) afforded *N*-cyclohexyl-2-ferrocenylpropanamide (VIb) as yellow needles,

m.p. 140—141°C (Found: C, 67.16; H, 7.67; mol. wt. 339 by mass spectrometry. $C_{19}H_{25}FeNO$ calcd.: C, 67.26; H, 7.43%. mol. wt., 339.27).

*6-(2-Ferrocenylacetamido)penicillanic acid sodium salt*

*(a). Preparation from the acid chloride.* Ferrocenylacetyl chloride (5.25 g, 0.02 mol) in dichloromethane (10 ml) was added dropwise to a mixture of 6-aminopenicillanic acid (4.32 g, 0.02 mol) and triethylamine (4.04 g, 0.04 mol) in dry dichloromethane (65 ml) at 2°C. The mixture was stirred for 1 h at 15°C, then filtered and the filtrate evaporated under vacuum at room temperature. The residue was dissolved in a mixture of water (100 ml) and ethyl acetate (50 ml) and the aqueous phase adjusted to pH 2.5 with 2 *M* HCl. The ethyl acetate layer was washed with water, dried ($MgSO_4$), filtered and a 40% solution of sodium 2-ethyl-hexanoate in 4-methylpentan-2-one (8.5 ml) added. The mixture was concentrated under vacuum and added dopwise to dry ether (600 ml). The sodium salt of 6-(2-ferrocenylacetamido)penicillanic acid, which separated as a pale yellow solid, was washed by decantation with ether and collected by filtration (6.74 g, 72%), m.p. 207—210°C (Decompn.) (Found: C, 50.25; H, 4.71; N, 5.74; $C_{20}H_{21}FeN_2NaO_4S \cdot H_2O$ calcd.: C, 49.81; H, 4.80; N, 5.81%). PMR (60 MHz) ($\delta$, $D_2O$): 5.49 (2H, s, methine at C5 and C6), 4.24 (9H, s, ferrocenyl), 4.20 (1H, s, methine at C3), 3.38 (2H, s, methylene), 1.60 (3H, s, methyl) and 1.51 (3H, s, methyl).

*(b). Preparation by direct condensation of ferrocenylacetic acid with 6-aminopenicillanic acid.* A mixture of ferrocenylacetic acid (1.22 g, 0.005 mol) and *N,N'*-dicyclohexylcarbodiimide (1.03 g, 0.005 mol) in dichloromethane (40 ml) was stirred at room temperature for 1 h. To the mixture was added a suspension of 6-aminopenicillanic acid (1.08 g, 0.005 mol) and triethylamine (0.51 g, 0.005 mol) in dichloromethane (20 ml). The reaction mixture was stirred for 3 h at room temperature and the precipitated dicyclohexylurea was removed by filtration. The filtrate was worked up as previously described to give 6-(2-ferrocenylacetamido)-penicillanic acid (Ie, $R^1$ = Na) (0.74 g, 30%). The IR and PMR spectra and m.p. were identical with the salt prepared by the acid chloride route. The 6-(2-ferrocenylacetamido)-penicillanic acid was characterised as the benzylamine salt (Ie, $R^1 = PhCH_2^+NH_3$), obtained as yellow crystals, m.p. 125—128°C (Decompn.) (Found: C, 58.61; H, 5.55; N, 7.44; $C_{27}H_{31}O_4FeN_3S$ calcd.: C, 59.02; H, 5.69; N, 7.65%).

The penicillins Id, If, Ig and Ih ($R^1$ = Na) were prepared from the acid chlorides III, IVb, IVc and V respectively as described above, and characterised as the amine salts Id ($R^1 = C_{10}H_{15}^+NH_3$), If and Ig ($R^1 = PhCH_2^+NH_3$) and If, Ig and Ih ($R^1 = C_6H_{11}^+NH_3$). The yield, m.p. and analyses are given in Table 2.

*7-(2-Ferrocenylacetamido)cephalosporanic acid sodium salt*

The reaction of 7-aminocephalosporanic acid (0.45 g, 0.00165 mol) with ferrocenylacetyl chloride (0.43 g, 0.00165 mol) was carried out as described for the preparation of the corresponding penicillin salt Ie ($R^1$ = Na). The product, 7-(2-ferrocenylacetamido)cephalosporanic acid (IIe; $R^1$ = Na), was obtained as a yellow solid, 0.51 g (59%), m.p. 168—170°C (dec.) (Found: C, 48.89; H, 4.54; N, 4.68; $C_{22}H_{21}FeN_2NaO_6S \cdot H_2O$ calcd.: C, 49.08; H, 4.31; N, 5.20%).

The cephalosporins IId, IIf and IIg ($R^1$ = Na) were prepared from the corresponding acid chlorides III, IVb and IVc as described above. The yield, m.p. and analyses are given in Table 2.

*7-(2-Ferrocenylacetamido)cephalosporanic acid adamantanamine salt*

The sodium salt of 7-(2-ferrocenylacetamido)cephalosporanic acid (0.1 g, 0.0002 mol) in water (50 ml) was layered with ether (50 ml) and the aqueous layer adjusted to pH 2.5 with 1 *M* HCl. The phases were separated and the organic layer washed with water, dried ($MgSO_4$), and filtered. Adamantanamine (0.03 g, 0.0002 mol) in ether (2 ml) was added dropwise to the ether solution of the cephalosporanic acid IIe ($R^1$ = H). The salt IIe ($R^1 = C_{10}H_{15}^{+}NH_3$) which separated, was washed by decantation with ether and collected by filtration to give orange crystals, m.p. 165—167°C (dec.) (Found: C, 58.86; H, 6.20; N, 6.25. $C_{32}H_{39}FeN_3O_6S$ calcd.: C, 59.17; H, 6.06; N, 6.47%).

The adamantanamine salts of the cephalosporins IIf and IIg ($R^1 = C_{10}H_{15}^{+}NH_3$) were prepared as described above. The yield, m.p. and analyses are given in Table 2.

## Acknowledgements

We thank Mr. G.O. Thomas of The Guest Hospital, Dudley for in vitro testing of the ferrocenyl-penicillins and -cephalosporins.

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