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Fatty peptides. VI. Penicillin and cephalosporin esters with increased lipophilic character

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Summary

Several series of methoxycarbonyl alkyl esters, **2b–d**, **3b** and **4b–d**, with increased lipophilicity, were prepared by conjugating penicillin G, ampicillin and cefuroxime, respectively. The suitability of this type of ester linkage as a pro-drug linkage for the carboxylic acid group of β-lactam antibiotics was determined both in vitro and in vivo. Only one compound, the penicillin G conjugate **2b** showed a weak activity in vitro against the sensitive *Staphylococcus aureus* strain (663E), *Pseudomonas* and clostridia. The remaining conjugates **2c**, **d**, **3b** and **4b–d** were inactive. Conjugates **2b–c** and **4b** exhibited antibiotic activity against *S. aureus* 663E following subcutaneous administration in the mouse. The most active conjugates **2b** and **4b** were methyl octanoyl derivatives of penicillin G and cefuroxime. It can be assumed that the secondary alcohol ester linkage was cleaved in vivo to afford active, presumably the parent, antibiotic. The penicillin G conjugate **2b–d** and the ampicillin conjugate **3b** were orally active, conjugate **3b** was more active than the parent ampicillin, and cefuroxime conjugates **4c** and **d** were orally inactive. Conjugation of a lipidic moiety via a secondary alcohol ester linkage may improve the absorption of β-lactam antibiotics. There appeared to be a preference for short alkyl chains for oral and subcutaneous activity in this series of conjugates, therefore, it can be assumed that the longer alkyl chains in compounds **2d**, **4c** and **d** protect the ester bond from esterases.

Introduction

Despite the outstanding clinical success of the β-lactam antibiotics, effective absorption of many of these compounds, particularly following oral administration, has plagued investigators in this field. Even compounds with appreciable activity after oral administration, such as ampicillin (**3a**), are not fully absorbed from the gastrointestinal tract (Kirby and Kind, 1967). An attempt to couple several neutral and acidic amino acids to phenoxymethylpenicillin and cephalothin has been reported (Bounkhala at al., 1988), and it was envisaged that the compounds might be better taken up by lysosomes than the parent. How-

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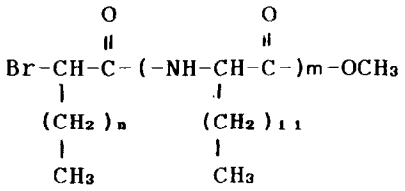
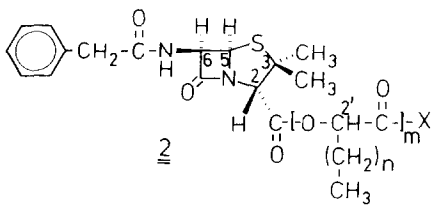


1

1	n	m
a	5	0
b	11	0
c	13	0
d	11	1



2

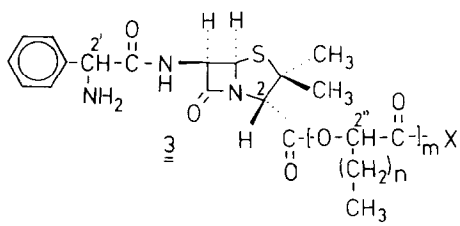
2	n	m	X
a	–	0	OH
b	5	1	OCH_3
c	11	1	OCH_3
d	13	1	OCH_3

ever, the conjugates were devoid of antimicrobial activity, presumably due to the stability of the conjugate towards degrading enzymes. The free carboxylic acid function of the penicillins and cephalosporins is necessary for antibiotic activity and larger mammals, including man, lack an esterase capable of hydrolysing simple β-lactam antibiotic esters (Agersborg at al., 1966). Nevertheless, it should be possible to conjugate lipidic units to the carboxylic acid, in the hope that enzymatic hydrolysis will give the active antibiotic in vivo. A degree of biological or chemical instability would, however, have to be built into the linking group.

Two types of ester groups suitable for linking lipidic amino acids and peptides were investigated: firstly acyloxyalkyl esters which are related to established enzymically labile pro-drug derivatives (Hughes at al., 1991), and secondly, novel methoxycarbonyl alkyl esters.

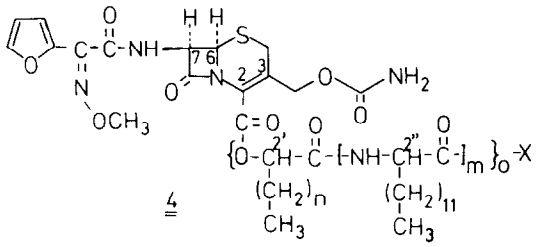
Materials and Methods

Infrared spectra were recorded with a Perkin Elmer 841 spectrophotometer. ^{1}H-NMR spectra were obtained on Varian XL-300 and Bruker AM500 instruments operating at fields of 300 and 500 MHz, respectively; chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were run on a VG Analytical ZAB-SE instrument, using fast atom bombardment (FAB) ionization. Reaction progress was monitored by thin-layer chromatography (TLC) on kieselgel PF_{254} using dichloromethane : methanol (10 : 1) as the mobile phase. Purification was achieved by flash chromatography through kieselgel G (dichloromethane : methanol 10 : 0.5). Solvents were evaporated under reduced pressure

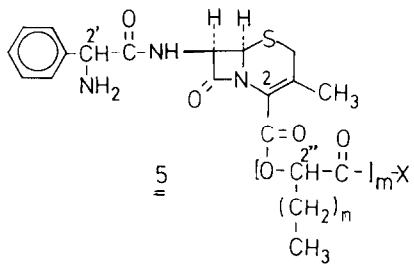


3

3	n	m	X
a	–	0	OH
b	5	1	OCH_3
c	13	1	OCH_3



4	n	m	o	X
a	–	–	0	OH
b	5	0	1	OCH_3
c	13	0	1	OCH_3
d	11	1	1	OCH_3



5	n	m	X
a	–	0	OH
b	5	1	OCH_3
c	13	1	OCH_3

TABLE 1

Summary of experimental data

Product	Starting compounds	Yield (%)	Analysis			
2b	**2a, 1a**	77	$C_{25}H_{34}N_2O_6S$ (490.6)			
			Calcd.	C 61.20	H 6.98	N 5.71
			Found	C 61.15	H 7.00	N 5.52
2c	**2a, 1b**	51	$C_{31}H_{46}N_2O_6S$ (574.8)			
			Calcd.	C 64.78	H 8.07	N 4.87
			Found	C 64.55	H 8.00	N 4.75
2d	**2a, 1c**	38	$C_{33}H_{50}N_2O_6S$ (602.8)			
			Calcd.	C 65.75	H 8.36	N 4.65
			Found	C 65.90	H 8.15	N 4.59
3b	**3a, 1a**	43	$C_{25}H_{35}N_3O_6S$ (505.6)			
			Calcd.	C 59.39	H 6.98	N 8.31
			Found	C 59.24	H 6.89	N 8.11
4b	**4a, 1a**	46	$C_{25}H_{32}N_4O_{10}S$ (580.6)			
			Calcd.	C 51.72	H 5.56	N 9.65
			Found	C 51.48	H 5.39	N 9.40
4c	**4a, 1c**	51	$C_{33}H_{48}N_4O_{10}S$ (692.8)			
			Calcd.	C 57.21	H 6.98	N 8.09
			Found	C 57.07	H 6.83	N 7.85
4d	**4a, 1d**	20	$C_{45}H_{71}N_5O_{11}S$ (890.1)			
			Calcd.	C 60.72	H 8.04	N 7.87
			Found	C 60.88	H 8.00	N 7.69

with a rotary evaporator. Melting points are not given for enantiomers. Analytical HPLC separation was carried out on a Whatman Partisil 5 RAC silica column. HPLC grade dichloromethane (Aldrich) and methanol (Rathburn) were filtered through a 25 μm membrane filter and degassed with helium flow prior to use. Separation was achieved with a solvent gradient beginning with 0% methanol, increasing constantly to 50% methanol at 15 min and decreasing steadily to 0% methanol from 17 to 20 min at a constant flow of 3 ml min^{-1}. The gradient was effected by two microprocessor-controlled Gilson 302 single-piston pumps. Compounds were detected with a Holochrome UV-Vis detector at 254 nm. Chromatographs were recorded with an LKB 2210 single-channel chart recorder. The experimental data are summarized in Table 1.

Measurement of in vitro activity

Minimum inhibitory concentrations (MICs) were determined by incorporation of compounds into Iso-Sensitest agar (Oxoid Ltd, U.K.). Final levels in the medium were in the range 125–0.06 mg/l. Aerobic test organisms were applied to the agar by multipoint inoculator (Denley Instrument Limited) at 10^3 and 10^7 colony forming units (cfu) per spot. For anaerobes, the medium was supplemented with 5% (v/v) defibrinated horse blood (Oxoid Ltd) and the organisms tested at a single inoculum of 10^5 cfu. Plates were incubated at 37°C for 24 h under aerobic and anaerobic conditions (Gaspak System, BBL) as appropriate. MICs were recorded as the lowest concentration completely inhibiting visible bacterial growth.

Experimental chemotherapy

Protection tests in mice were performed according to literature methods (Ryan at al., 1976), using a non-penicillinase producing strain of *Staphylococcus aureus* (strain 663E). Female CD1 mice (18–20 g) were challenged intraperitoneally with approx. 10 times the 50% lethal dose (1.25×10^6 cfu/mouse) of bacteria, suspended in 0.5 ml of brain-heart infusion broth containing a final concentration of 1.5% dried baker's yeast to potentiate virulence. Compounds were dissolved initially in dimethyl sulphoxide (final concentration 10%), followed by serial 4-fold dilutions in 0.5% sodium carboxymethylcellulose containing 10% DMSO. Dose levels generally ranged from 25 to 0.4 mg/kg. Five mice were used at each of the compound dose concentrations and dosing (0.2 ml volume) was administered either subcutaneously (s.c.) or orally (p.o.) at 1 and 5 h post-challenge. The median effective dose (ED_{50} mmol/kg per dose) was calculated by logit transformation from the number of animals surviving at each dose level on day 5.

*Methyl N-(2-bromotetradecanoyl)-2-aminotetradecanoate (**1d**)*

2-Bromotetradecanoic acid (1.150 g, 3.40 mmol) and methyl 2-aminotetradecanoate (1.000 g, 3.40 mmol) were reacted as described by Gibbons et al. (1990) to yield: 0.843 g (45%). ^{1}H-NMR ($CDCl_3$): δ = 6.77 (1H,t,CONH), 4.57 (1H,m,Br-CH), 4.32 (1H,m,α-CH), 3.76 (3H,s,$COOCH_3$), 2.08 (2H,bm,β-CH_2), 1.88 (2H,bm,β-CH_2), 1.69 (2H,bm,CH_2), 1.37 (2H,bm,γ-CH_2), 1.27 (36H,m,18xCH_2), 0.88 (6H,t,2xCH_3). MS *m/z* (%) = 601 (17), 548 (16), 546 (20), $[M + H]^+$, 488 (8), 486 (9), 466 (9), 258 (20), 198 (100), 95 (13), 85 (15), 69 (25), 57 (24), 55 (36).

*[2'(S,R),2S,5R,6R] (Methyl octanoate)-2'-yl 6-phenylacetamido-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylate (**2b**)*

Penicillin-G (**1b**) potassium salt (1.34 mmol) and 18-crown-6 (354 mg, 1.34 mmol) were stirred in a mixture of ethanol (10 ml) and water (2 ml) for 1 h at room temperature. The solvents were removed in vacuo at 30°C and the residue lyophilised overnight. The resultant cefuroxime crown-ether complex was added to methyl 2-bromooctanoate (**1a**) (320 mg, 1.34 mmol) in dimethyl formamide (5 ml) and the solution stirred overnight at room temperature. After dilution with ethyl acetate (50 ml) and washing with brine (50 ml), 3% sodium hydrogen carbonate (50 ml) and brine (50 ml), the organic phase was dried (Na_2SO_4) and evaporated. The crude material was purified by TLC. ^{1}H-NMR($CDCl_3$): δ = 7.35 (5H,m,aromatic H), 5.65 (1H,m,C_6-H), 5.50 (1H,m,C_5-H), 5.00 (1H,2t,α-CH), 4.50, 4.40 (1H,s,C_2-H), 3.75 (3H,s,$COOCH_3$), 3.65

(2H,s,benzyl CH_2), 1.90 (2H,m,β-CH_2), 1.58, 1.50, 1.42 (6H,3s,2xCH_3), 1.30 (8H,m,4xCH_2), 0.80 (3H,t,CH_3). MS *m/z* (%) = 513[M + Na]$^+$ (100), 338 (19), 316 (87), 279 (5), 215 (4), 160 (6), 114 (5), 91 (36), 55 (13), 39 (5).

[2'-(S,R),2S,5R,6R] (Methyl tetradecanoate)-2'-yl 6-phenylacetamido-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate ***(2c)***

^{1}H-NMR($CDCl_3$): δ = 7.38, 7.34, 7.30 (5H,m,aromatic H), 6.06 (1H,t,CONH), 5.67 (1H,m,C_6-H), 5.51 (1H,m,C_5-H), 5.06, 5.01 (1H,2t,α-CH), 4.50, 4.40 (1H,2s,C_2-H), 3.75 (3H,m,$COOCH_3$), 3.66 (2H,m,benzyl CH_2), 1.89 (2H,m,β-CH_2), 1.58, 1.50, 1.44 (6H,3s,2xCH_3), 1.28 (20H,m,10xCH_2), 0.90 (3H,t,CH_3). MS *m/z* (%) = 620 (69) [M + 2Na − H]$^+$, 597 (100) [M + Na]$^+$, 422 (17), 400 (49), 176 (37), 114 (15), 91 (20).

[2'-(S,R),2S,5R,6R] (Methyl hexadecanoate)-2'-yl 6-phenylacetamido-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate ***(2d)***

^{1}H-NMR($CDCl_3$): δ = 7.34 (5H,m,aromatic H), 6.05 (1H,m,CONH), 5.65 (1H,quintet,C_6-H), 5.50 (1H,t,C_5-H), 5.03 (1H,m,α-CH), 4.47, 4.40 (1H,2s,C_2-H), 3.74 (3H,m,$COOCH_3$), 3.64 (2H,m,benzyl CH_2), 1.87 (2H,m,β-CH_2), 1.52, 1.44 (6H,2s,2xCH_3), 1.27 (24H,m,12xCH_2), 0.89 (3H,t,CH_3). MS *m/z* (%) = 647 (100) [M + 2Na − H]$^+$, 626 (35), 625 (91) [M + Na]$^+$, 450 (23), 428 (52), 391 (5), 274 (5), 176 (8), 114 (22), 91 (25), 69 (9), 55 (10).

[2'-(S,R),2'R,2S,5R,6R] (Methyl octanoate)-2'-yl 6-(2'-amino-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate ***(3b)***

^{1}H-NMR($CDCl_3$): δ = 7.44 (2H,m,NH_2), 7.36, 7.30 (5H,m,aromatic H), 5.67 (1H,m,C_6-H), 5.51 (1H,m,C_5-H), 5.14 (1H,m,CONH), 5.04, 4.65 (2H,2m,2xα-CH), 4.59 (1H,d,C_2-H), 3.75 (3H,s,$COOCH_3$), 1.87 (2H,m,β-CH_2), 1.55, 1.45 (6H,2s,2xCH_3), 1.26 (8H,m,4xCH_2), 0.90 (3H,t,CH_3). MS *m/z* (%) = 550 (16) [M + 2Na − H]$^+$, 528 (5) [M + Na]$^+$, 338 (35), 316 (49), 279 (10), 199 (13), 176 (75), 173 (48), 160 (15), 113 (95), 106 (100), 93 (24), 87 (27), 83 (18), 69 (16), 55 (43).

[2'-(S,R),6R,7R] (Methyl octanoate)-2'-yl 3-carbamoyloxymethyl-7-[furan-2-yl(hydroxylimino)-acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***(4b)***

^{1}H-NMR($CDCl_3$): δ = 7.50 (1H,d,CONH), 6.91 (1H,d,C_5'-H), 6.50 (2H,m,C_3'-H,C_4'-H), 6.00, 5.14 (2H,ab,m,OCH_2), 5.81 (1H,m,C_7-H), 5.35 (1H,m,C_6-H), 5.08 (1H,m,α-CH), 4.70 (2H,m,$CONH_2$), 4.08 (3H,s,$NOCH_3$), 3.78 (3H,s,$COOCH_3$), 3.64, 3.48 (2H,m,2xCH), 1.89 (2H,m,β-CH_2), 1.31 (8H,m,4xCH_2), 0.90 (3H,t,CH_3). MS *m/z* (%) = 603 (100) [M + Na]$^+$, 542 (5), 326 (9), 199 (12), 176 (50), 173 (33), 92 (18).

[2'-(S,R),6R,7R] (Methyl hexadecanoate)-2'-yl 3-carbamoyloxymethyl-7-[furan-2-yl (hydroxylimino) acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***(4c)***

^{1}H-NMR($CDCl_3$): δ = 7.53 (1H,m,CONH), 6.91 (1H,d,C_5'-H), 6.54 (1H,d,C_3'-H), 5.49 (1H,m,C_4'-H), 5.86, 5.43 (2H,m,C_6-H,C_7-H), 5.26 (2H,m,$CONH_2$), 5.12 (2H,m,OCH_2), 4.98 (1H,m,α-CH), 4.11 (3H,s,$NOCH_3$), 3.75 (3H,s,$COOCH_3$), 3.72 (2H,m,2xCH), 1.98 (2H,m,β-CH_2), 1.76 (2H,m,CH_2), 1.27 (22H,m,11xCH_2), 0.88 (3H,t,CH_3). MS *m/z* (%) = 715 (100) [M + Na]$^+$ 413 (5), 329 (9), 199 (10), 176 (73), 136 (11), 92 (10), 77 (8), 55 (8).

[2'-(S,R),2''(S,R),6R,7R] [(Methyl tetradecanoate)-2''-yl-tetradecanoylamido]-2'-yl 3-carbamoyloxymethyl-7-[furan-2-yl (hydroxylimino) acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate ***(4d)***

^{1}H-NMR($CDCl_3$): δ = 7.51 (1H,m,CONH), 6.92 (1H,d,C_5'-H), 6.54 (1H,d,C_3'-H), 6.49 (1H,q,C_4'-H), 5.86, 5.36 (2H,m,C_6-H,C_7-H), 5.26 (1H,m,CONH), 5.13 (2H,m,OCH_2), 5.00, 4.50 (4H,m,2xα-CH,$CONH_2$), 4.10 (3H,s,$NOCH_3$), 3.75 (3H,s,$COOCH_3$), 3.72 (2H,m,C_2-H_2), 1.98 (4H,m,2xβ-CH_2), 1.67 (4H,m,2x-CH_2), 1.26 (36H,m,18xCH_2), 0.88 (6H,t,2xCH_3). MS *m/z* (%) = 913 (100) [M + Na]$^+$ 868 (4), 704 (5), 506

(8), 447 (5), 334 (12), 258 (7), 198 (13), 124 (13), 67 (16), 55 (27).

Results and Discussion

Chemistry

Methoxycarbonyl alkyl esters of the carboxylic acid group of β-lactam antibiotics are novel derivatives whose stability in biological systems was not known. Thus, as an example of a different type of potentially labile linkage, lipidic conjugates were prepared from the β-lactam antibiotics penicillin G (**2a**), ampicillin (**3a**) and cefuroxime (**4a**), using 2-bromo alkanoic acid methyl esters **1a–c** and the dimer **1d**. The esters **1a–c** were prepared from the corresponding acids with thionyl chloride in methanol. The dimeric lipidic peptide conjugating unit **1d** was obtained by condensing methyl 2-aminotetradecanoate (Gibbons et al., 1990) to 2-bromotetradecanoic acid, using standard solution-phase peptide synthetic methods. The lipidic ester conjugate **2b** was synthesized by coupling the bromomethyl ester **1a** to the sodium salt of penicillin G (**2a**) using a crown-ether assisted coupling method (Hughes at al., 1991). Firstly, a complex of the sodium salt of penicillin G (**2a**) with the macrocyclic ether, 18-crown-6 was prepared, then the complex was reacted with the bromomethyl ester **1a**, furnishing the ester conjugate **2b** in good yield. This same procedure was used to synthesise the other penicillin G conjugates **2c** and **2d**, the ampicillin conjugate **3b** and the cefuroxime conjugates **4b–d**.

Biology

In vitro and in vivo activity of methoxycarbonyl alkyl esters

The methoxycarbonyl alkyl esters **2b–d**, **3b** and **4b–d** were compounds of unknown stability in biological systems. The suitability of the methoxycarbonyl alkyl ester group as a pro-drug linkage for the carboxylic acid group of β-lactam antibiotics was thus determined using in vitro and in vivo experiments.

The in vitro activities of methoxycarbonyl alkyl ester **2b–d**, **3b** and **4b–d** were determined against the same organisms used for the amide conjugates (Toth at al., 1991) (Table 2). Conjugate **2b** exhibited antibiotic activity against the sensitive *S. aureus* strain (663E), *Pseudomonas* and clostridia. The results were consistent with a partial hydrolysis of the penicillin G conjugate **2b**, thus liberating some free penicillin G (**2a**) during the course of the experiment. The remaining compounds **2c**, **d**, **3b** and **4b–d** were inactive as expected.

The in vivo activity of the methoxycarbonyl

TABLE 2

Results of in vitro tests (mmol / l × 10^{-3}) of methoxycarbonyl alkyl ester conjugates of β-lactam antibiotics [H = high (10^{7} cfu / ml) inoculum; L = low (10^{5} cfu / ml) inoculum]

Compound	*S. aureus* [a] 1033E	*S. aureus* [b] 663E	*E. coli* 1852E	*Ps. aeruginosa* 2033E	*C. perfringens* 2045E
2a H	> 336	0.16	43	0.67	
L	5.37	0.16	21.5	0.35	21.5
2b H	> 255	8.16	> 255	126	
L	> 255	4.08	> 255	16.3	32.6
3a L	0.15	0.15	0.32	0.15	0.15
4a H	> 294	0.59	2.35	2.35	
L	72.9	0.30	0.30	1.17	4.70

[a] β-Lactamase-producing.
[b] Non-β-lactamase-producing.

TABLE 3

Comparison of in vitro and in vivo (s.c. and p.o). activity of β-lactam antibiotic methoxycarbonyl alkyl ester

Conjugates against *S. aureus* 663E (ED_{50}: dose required to protect 50% of animals from lethal infection)

Compound	In vivo ED_{50} (mg/kg × 2)	
	s.c.	p.o.
2a	1.34	9.67
2b	2.67	11.20
2c	< 9.23	9.23
2d	ia [a]	27.40
3a	3.22	3.22
3b	ndr	2.52
4a	8.47	58.80
4b	4.13	ndr
4c	ia [b]	ia [b]
4d	ia [c]	ia [c]

ia: inactive at dose equivalent to [a] 41 mmol/kg, [b] 36 mmol/kg, [c] 28 mmol/kg of active drug. ndr: no dose response.

alkyl ester conjugates **2b–d**, **3b** and **4b–d** against *S. aureus* 663E was determined after subcutaneous and oral administration (Table 3).

The penicillin and conjugates **2b**, **c** and the cefuroxime conjugate **4b** exhibited high antibiotic activity following subcutaneous administration. Therefore, it can be assumed that the secondary alcohol ester linkage (methoxycarbonylalkyl ester) is cleaved in vivo to afford active, presumably the parent antibiotic. The ampicillin conjugate **3b** did not elicit a dose-dependent response in the test. The other three compounds tested were inactive. There appeared to be a preference for short alkyl chains for subcutaneous activity in this series of conjugates. Thus, the most active conjugates **2b** and **4b** were methyl-octanoyl derivatives of penicillin G (**2a**) and cefuroxime (**4a**), respectively, whereas the longer chain derivative of penicillin G, **2c**, was somewhat less active. Also, the penicillin and conjugate **2d**, and the cefuroxime derivatives **4c** and **4d** were inactive. The longer alkyl chains in compounds **2d**, **4c** and **d** may protect the ester bond from esterases.

The penicillin G conjugate **2b–d** and the ampicillin conjugate **3b** were active when given orally. The cefuroxime conjugate **4b** did not show a dose-dependent response during the experiment. Cefuroxime conjugates **4c** and **d** were orally inactive. The antibiotic activity of the methoxycarbonyl alkyl ester **3b**, when given by the oral route, was better than that of the parent ampicillin (**3a**). Lipidic conjugation via a bioreversible ester linkage was able to impart a positive effect on the absorption of **3b** ampicillin conjugate. Penicillin G conjugates **2b** and **c** showed similar oral activity to that of the penicillin G (**2a**). It is not unreasonable to assume that these conjugates were hydrolysed in the gut of the test animals to yield the active and absorbable parent compounds.

Conclusion

Several series of methoxycarbonyl alkyl esters, **2b–d**, **3b** and **4b–d** with increased lipophilicity, were prepared by conjugating penicillin G, ampicillin and cefuroxime, respectively. Only one compound, conjugate **2b**, showed a weak activity in vitro against the sensitive *S. aureus* strain (663E), *Pseudomonas* and clostridia. The remaining conjugates **2c**, **d**, **3b** and **4b–d** were inactive, as expected. Conjugates **2b**, **c** and **4b** exhibited antibiotic activity against *S. aureus* 663E following subcutaneous administration in the mouse. Therefore, it can be assumed that the secondary alcohol ester linkage is cleaved in vivo to afford active, presumably the parent, antibiotic. There appeared to be a preference for shorter alkyl chains for subcutaneous activity in this series of conjugates. The longer alkyl chains in compounds **2d**, **4c** and **d** may protect the ester bond from esterases.

The penicillin G conjugates **2b–d** and the ampicillin conjugate **3b** were active when given orally; cefuroxime conjugates **4c** and **d** were inactive. The antibiotic activity of **3b**, was better than that of the parent ampicillin (**3a**). Lipidic conjugation via a bioreversible ester linkage was able to impart a positive effect on the absorption of **3b** ampicillin conjugate. Penicillin G conjugates **2b** and **c** showed similar oral activity to that of penicillin G (**2a**). It is not unreasonable to assume that the latter conjugates were hydrolysed

in the gut of the test animals to yield the active and absorbable parent compound.

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