International Journal of Pharmaceutics, 73 (1991) 259–266

ADONIS 037851739100268P

IJP 02464

Lipidic peptides. IV. Penicillin and cephalosporin amide conjugates with lipidic amino acids and their oligomers

Istvan Toth *, Richard A. Hughes, Peter Ward [1], Michael A. Baldwin, Kevin J. Welham, Andrew M. McColm [1], David M. Cox [1] and William A. Gibbons

The School of Pharmacy, University of London, 29–39 Brunswick Sq., London WC1N 1AX (U.K.) and [1] Glaxo Group Research Ltd, Greenford Road, Greenford, Middlesex UB6 0HE (U.K.)

(Received 4 February 1991)
(Modified version received 10 March 1991)
(Accepted 21 March 1991)

Key words: Lipidic amino acid; Lipidic peptide; Transmembrane drug delivery; Antibiotic; Penicillin; Cephalosporin

Summary

A series of lipidic amide conjugates (**2b, c, 3b, c, 4b-d, 5b** and **c**) of β-lactam antibiotics were synthesised using mixed anhydride methods to couple the Boc-protected lipidic amino acids (**1a** and **b**) and oligomer (**1c**) to a variety of penicillins and cephalosporins. Conjugates (**2b, c, 3b, 4b-d** and **5b**) showed weak to moderate activity in vitro and were only weakly active in vivo against the non-β-lactamase producing strain *S. aureus 663E*.

Introduction

Fatty amino acids are α-amino acids with a linear or branched alkyl side chain. A number of uses can be envisaged that exploit the amphipathic nature of these compounds and their oligomers. Of particular interest is the use of fatty amino acids monomers and oligomers (Gibbons et al., 1990) as conjugating units for biologically active compounds. The conjugates formed would be expected to possess a degree of lipid- or membrane-like character due to the long alkyl side chains of the lipidic moieties. It is anticipated that this feature will enhance the passage of poorly absorbed drugs across biological membranes to reach their site of action.

Because of their bifunctional nature, the lipidic amino acids and peptides have the capacity to be chemically conjugated to drugs with a wide variety of functional groups. The linkage between the drug and the lipidic unit may either be biologically stable (i.e., a new drug is formed) or possess predictable biological or chemical instability (i.e., the conjugate is a pro-drug). In either case, the resulting conjugates could possess a high degree of membrane-like character, sufficient to facilitate their passage across membranes. The long

Correspondence: W.A. Gibbons, The School of Pharmacy, University of London, 29–39 Brunswick Sq., London WC1N 1AX, U.K.
* On leave from the Central Research Institute for Chemistry of the Hungarian Academy of Sciences.

hydrocarbon side chains may also have the additional effect of protecting a labile parent drug from enzymatic attack and hence promote metabolic stability and reduce the required dose.

In this paper, we report investigation into the ability of lipidic amino acids and peptides to improve the oral absorption of the β-lactam antibiotics.

Despite the outstanding clinical success of the β-lactam antibiotics, ineffective absorption of these compounds, particularly following oral administration, has continually plagued investigators in this field. Even compounds that show appreciable activity after oral administration, such as α-amino benzyl penicillin (ampicillin, **3a**) are by no means fully absorbed from the gastro-intestinal tract (Kirby and Kind, 1967).

There are several possibilities for conjugation of lipidic amino acids and peptides to the β-lactam antibiotics, by conjugation through either the free carboxylic or the free amino functions. A number of β-lactam antibiotics possess a free amino function which may be acylated with *N*-protected lipidic amino acids, which should provide a convenient way of introducing lipidic functionality to antibiotics.

Materials and Methods

Infra-red 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) ionisation. 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 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 summarised in Table 1.

TABLE 1

Synthesis of β-lactam conjugates

Product	Starting compounds	Yield (%)	Analysis
2b	**1a, 2a**	58.3	$C_{23}H_{39}N_3O_6S$ (485.6) Calcd. C, 56.88; H, 8.09; N, 8.65 Found C, 56.51; H, 8.33; N, 8.29
2c	**1b, 2a**	55	$C_{27}H_{47}N_3O_6S$ (541.7) Calcd. C, 59.86; H, 8.75; N, 7.76 Found C, 59.59; H, 8.87; N, 7.55
3b	**1a, 3a**	80	$C_{31}H_{46}N_4O_7S$ (618.8) Calcd. C, 60.17; H, 7.49; N, 9.05 Found C, 60.32; H, 7.49; N, 8.81
3c	**1b, 3a**	67	$C_{35}H_{54}N_4O_7S$ (674.9) Calcd. C, 62.28; H, 8.04; N, 8.30 Found C, 62.00; H, 8.39; N, 8.21
4b	**1a, 4a**	63	$C_{25}H_{39}N_3O_8S$ (541.7) Calcd. C, 55.42; H, 7.26; N, 7.76 Found C, 55.29; H, 7.41; N, 7.58
4c	**1b, 4a**	69	$C_{29}H_{47}N_3O_8S$ (597.8) Calcd. C, 58.26; H, 7.92; N, 7.03 Found C, 58.00; H, 7.99; N, 6.77
4d	**1c, 4a**	47	$C_{35}H_{58}N_4O_9S$ (710.9) Calcd. C, 59.13; H, 8.22; N, 7.88 Found C, 58.97; H, 8.39; N, 7.65
5b	**1a, 5a**	65	$C_{31}H_{44}N_4O_7S$ (616.8) Calcd. C, 60.36; H, 7.19; N, 9.08 Found C, 60.11; H, 7.33; N, 8.85
5c	**1b, 5a**	67	$C_{35}H_{52}N_4O_7S$ (672.9) Calcd. C, 62.47; H, 7.79; N, 8.33 Found C, 62.41; H, 7.86; N, 8.18

Synthesis

*[2'(S,R),2S,5R,6R]-6-[2'-(tert-Butoxycarbonylamino)-decanamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**2b**).* ^{1}H NMR ($CDCl_3$): δ = 7.25(1H,m,NH), 6.64(1H,m,C_6–H), 5.56(1H,m,C_5–H), 5.20(1H,M,NH), 4.45(1H,2s,C_2–H), 4.18(1H,m,α–CH), 1.82(2H,m,CH_2), 1.70, 1.68(3H,2s,CH_3), 1.62, 1.60(3H,2s,CH_3), 1.45(9H,s,C(CH_3)$_3$), 1.30(12H,m,6CH_2), 0.90(3H,t,CH_3). MS *m/z* (%) = 486 $(M + H)^+$ (55), 460 (24), 430 (13), 386 (18), 271 (13), 185 (14), 114 (10), 75 (15), 57 (100), 45 (20), 41 (26), 29 (23).

*[2'(S,R),2S,5R,6R]-6-[2'-(tert,-Butoxycarbonylamino)-tetradecanamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**2c**).* ^{1}H NMR ($CDCl_3$): δ = 8.40 (1H,br.,COOH), 7.38 (1H,br.,CONH), 5.58, 5.53 (2H,ab,C_5–H,C_6–H), 5.34 (1H,s,OCONH),4.40 (1H,d,C_2–H), 4.16 (1H,br.,α–CH), 1.77 (1H,m,β–CH), 1.65, 1.58 (2H,2s,2CH_3), 1.40 (9H,s,C(CH_3)$_3$), 1.24 (20H,s,10CH_2), 0.90 (3H,t,CH_3). MS *m/z* (%) = 586 $(M + 2Na–H)^+$ (11), 564 $(M + Na)^+$ (8), 204 (50), 198 (16) 182 (11), 174 (10), 160 (10), 119 (10), 88 (18), 79 (15), 72 (13), 63 (20), 57 (100).

*[2''(S,R),2'R,2S,5R,6R]-6-{2'-[2''-(tert-Butoxycarbonylamino)-decanamido]phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**3b**).* ^{1}H NMR ($CDCl_3$): δ = 7.70 (1H,m,NH), 7.26, 7.20 (5H,m,aromatic H), 5.65 (1H,m,NH), 5.47 (1H,m,CH), 5.40 (1H,m,C_5–H), 4.40(1H,m,C_2–H), 4.25, 4.05 (2H,2m,α–CH), 1.80(2H,m,CH_2), 1.63, 1.57 (6H,s,2CH_3), 1.50(9H,s,C(CH_3)$_3$), 1.35 (12H,m,6CH_2), 0.9(3H,t,CH_3). MS *m/z*(%) = 619$(M + H)^+$ (76), 593 (38), 563 (45), 519 (100), 471 (44).

*[2''(S,R),2'R,2S,5R,6R]-6-{2'-[2''-(tert-Butoxycarbonylamino)-tetradecanamido]-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid (**3c**).* ^{1}H NMR ($CDCl_3$): δ = 6.80 (1H,m,CONH), 7.35, 7.30 (5H,m,aromatic H), 5.60, 5.45 (3H,m,C_6–H,C_5–H,NH), 4.40 (1H,M,C_2–H), 4.25, 4.10 (2H,m,2β–CH), 1.80 (2H,m,CH_2), 1.55, 1.45 (6H,2s,2CH_3), 1.40 (9H,s,C(CH_3)$_3$), 1.25 (20H,m,10CH_2), 0.90 (3H,t,CH_3). MS *m/z* (%) = 720 $(M + 2Na–H)^+$ (4), 697 $(M + Na)^+$ (6), 287 (6), 204 (14), 198 (16), 160 (8), 106 (100), 74 (10), 57 (63).

*[2'(S,R),6R,7R]-3-Acetoxymethyl-7-[2'-(tert-butoxycarbonylamino)decanamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**4b**).* ^{1}H NMR ($CDCl_3$): δ = 5.77 (1H,m,CONH), 5.54–4.85 (5H,m,C_7–H,C_6–H,CH_2O, OCONH), 4.22 (1H,m,α–CH), 3.59–3.33 (2H,m,C_4–H_α,C_4–H_β), 2.01 (3H,s,$COCH_3$), 1.79 (1H,m,CH), 1.59 (1H,m,CH), 1.43 (9H,s,C(CH_3)$_3$), 1.24 (12H,m,6CH_2), 0.88 (3H,t,CH_3). MS *m/z* (%) = 586 $(M + 2Na–H)^+$ (3), 526 (12), 492 (10), 426 (20), 307 (28), 285 (12), 241 (11), 232 (11), 214 (13), 202 (21), 142 (15), 119 (10), 105 (42), 88 (21), 78 (11), 72 (15), 63 (20), 57 (100).

*[2'(S,R),6R,7R]-3-Acetoxymethyl-7-[2'-(tert-butoxycarbonylamino)tetradecanamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**4c**).* ^{1}H NMR ($CDCl_3$): δ = 5.77 (1H,m,CONH), 5.68–4.85 (5H,m,C_7–H,C_6–h,CH_2O,OCONH), 4.27 (1H,m,α–CH), 3.54–3.31 (2H,m,C_4–H_α,C_4–H_β), 2.01 (3H,s,$COCH_3$), 1.83 (1H,m,CH), 1.60 (1H,m,CH), 1.44 (9H,s,C(CH_3)$_3$), 1.25 (20 H,m,10 CH_2), 0.85 (3H,t,CH_3). MS *m/z* (%) = 642 $(M + 2Na–H)^+$ (5), 582 (15), 548 (11), 513 (17), 482 (17), 438 (8), 388 (17), 307 (38), 287 (38), 241 (12), 214 (15), 201 (24), 198 (34), 175 (11), 173 (14), 119 (14), 105 (41), 88 (21), 71 (13), 63 (19), 57 (100).

*[2'(S,R),2''(S,R),6R,7R]-3-Acetoxymethyl-7-{2'-[2''-(tert-butoxycarbonylamino)decanamido]decanamido}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**4d**).* ^{1}H NMR ($CDCl_3$): δ = 6.80, 5.68 (2H,2m,2 CONH), 5.47–4.84 (5H,m,C_7–H,C_6–H,OCH_2,OCONH), 4.45, 4.23 (2H,2m,2α–CH), 4.14–3.32 (2H,m,C_4–H_α,C_4–H_β), 2.10 (3H,d,$COCH_3$), 1.45 (9H,s,C(CH_3)$_3$), 1.83 (1H,m,CH), 1.60 (1H,m,CH), 1.26 (24H,m,12CH_2), 0.86 (3H,t,CH_3). MS *m/z* (%) = 778 $(M + 3Na–2H)^+$ (10), 501 (29), 479 (19), 449 (13), 424 (31), 402 (100), 379 (20), 173 (10), 142 (21), 57 (30).

*[2'(R),2''(S,R),6R,7R]-7-{2'-[2''-(tert-Butoxycarbonylamino)decanamido]phenylacetamido}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**5b**).* ^{1}H NMR ($CDCl_3$): δ = 7.35, 6.98 (5H,m,aromatic H), 5.84, 5.56

(2H,2m,2CONH), 5.67, 4.95 (2H,2m,C_7–H,C_6–H), 5.14 (1H,m,OCONH), 4.39, 4.16 (2H,2m,2α–CH), 3.49, 3.17 (2H,2q,C_4–H_α,C_4–H_β), 2.16 (3H,s,C_3–CH_3), 1.42 (9H,m,$C(CH_3)_3$), 1.18 (12H,m,6CH_2), 0.88 (3H,m,CH_3). MS *m/z* (%) = 661 $(M + 2Na–H)^+$ (8), 639 $(M + Na)^+$ (8), 627 (17), 583 (12), 561 (21), 539 (16), 470 (12), 448 (23), 444 (24), 422 (45), 390 (16), 364 (18), 342 (17), 323 (31), 298 (13), 279 (22), 257 (32), 232 (58), 202 (77), 173 (100), 158 (58), 119 (16), 92 (49), 88 (54), 72 (29), 63 (50), 57 (77).

[2'(R),2"(S,R),6R,7R]-7-{2'-[2"-(tert-Butoxycarbonylamino)tetradecanamido]phenylacetamido}3-methyl-7-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (5c). ^{1}H NMR ($CDCl_3$): δ = 7.35, 6.98 (5H,m,aromatic H), 5.82, 5.55 (2H,2m,2CONH), 5.67, 5.45 (2H,2m,C_7–H,C_6–H), 5.14 (1H,m,OCONH), 4.39, 4.15 (2H,2m,2α–CH), 3.46, 3.16 (2H,2q,C_4–H_α, C_4–H_β), 2.14 (3H,s,C_3–CH_3), 1.46 (9H,m,$C(CH_3)_3$), 1.23 (20 H,m,10CH_2) 0.88 (3H,t,CH_3). MS *m/z* (%) = 717 $(M + 2Na–H)^+$ (13), 695 $(M + Na)^+$ (26), 617 (17), 595 (15), 504 (21), 500 (19), 478 (45), 446 (11), 420 (15), 335 (11), 313 (22), 287 (29), 202 (77), 198 (26), 177 (22), 158 (67), 106 (65), 88 (39), 57 (100).

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 of 125 to 0.06 mg/l. Aerobic test organisms were applied to the agar by multipoint inoculator (Denley Instrument Ltd) 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 similarly to literature methods (Ryan et al., 1976), using a non-penicillinase producing strain of *Staphylococcus aureus* (strain *663E*). Female CD1 mice (18–20 g) were challenged intraperitoneally with approximately 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 dimethylsulphoxide (final concentration 10%), followed by serial 4-fold dilutions in 0.5% sodium carboxymethyl cellulose 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 h and 5 h post-challenge. The median effective dose (ED_{50} mg/kg/dose) was calculated by logit transformation from the number of animals surviving at each dose level on day 5.

Results and Discussion

Synthesis of β-lactam antibiotic conjugates

Four antibiotics with free amino groups, namely 6-amino penicillanic acid (6-APA) (**2a**) (Batchelor et al., 1959) and ampicillin (**3a**) (penicillins), and 7-amino cephalosporanic acid (7-ACA, **4a**) (Abraham and Newton, 1961) and cephalexin (**5a**) (cephalosporins) were acylated with lipidic amino acids and peptides (**1a–c**).

Several procedures for the acylation of amino-containing β-lactam antibiotics are known (Perron et al., 1960; Loder et al., 1961; Leanza et al., 1965; Bamberg et al., 1967; Ryan et al., 1969). A mixed anhydride method (Doyle et al., 1962) was used for the preparation of the 6-APA/lipidic amino acid conjugate **2b**. Compounds **2c**, **3b** and **3c** were prepared using analogous conditions, starting from either 6-APA (**2a**) or ampicillin (**3a**), and the Boc-protected lipidic amino acids **1a** and **1b** respectively.

Due to the poor solubility of cephalosporins in aqueous solvents, the cephalosporin derivatives **4b–d**, **5b** and **5c** were synthesised by an alternative mixed anhydride acylation procedure in organic solvents (Spencer et al., 1966) from either

7-ACA (**4a**) or cephalexin (**5a**) with the appropriate amino acids **1a**, **1b** and **1c**.

In vitro antibiotic activity

The minimum inhibitory concentration (MIC) of the compounds was determined in vitro against a variety of Gram positive and negative bacteria (Table 2).

Most compounds showed moderate to good activity against a non-penicillinase-producing strain of *Staphylococcus aureus*. This is interesting from a structure–activity viewpoint, in that conjugates **2b**, **c**, and **4b–d** do not have an aromatic side chain and very few β-lactam antibiotics with acyclic side chains have been reported. However, even the most active compounds, **2b**, **3b** and **4b** were an order of magnitude less potent than the antibiotics ampicillin (**3a**), penicillin G (**6**) and cefuroxime (**7**). The ampicillin conjugate **3b** showed activity against a β-lactamase-producing strain of *S. aureus* comparable to that of penicillin G, but significantly weaker than that of ampicillin (**3a**). The remaining conjugates were weakly active or inactive against this organism.

Four conjugates, **2b**, **3b**, **c** and **5b**, were active against *Escherichia coli*. Of these compounds, the ampicillin conjugates **3b** and **3c** were as potent as penicillin G (**6**). However, lipidic amino acid conjugation considerably reduced the activity of conjugates **3b** and **3c** relative to the unconjugated parent compound, ampicillin (**3a**). Conjugates **3b** and **3c** also demonstrated antibiotic activity against a sensitive strain of *Pseudomonas aeruginosa* being roughly equipotent with penicillin G (**6**), but two orders of magnitude less than ampicillin (**3a**).

All compounds except two, the 7-ACA conjugate **4c** and the cephalexin conjugate **5c** were

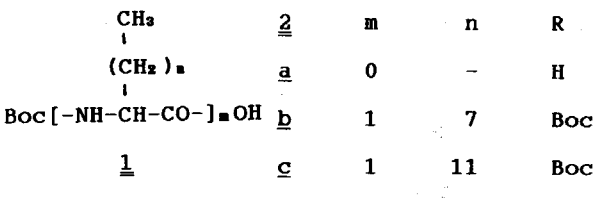
TABLE 2

*In vitro tests (mmol / l × 10^{-3}) of amide conjugates of β-lactam antibiotics and the unconjugated antibiotics ampicillin, penicillin G and cefuroxime (H = high (10^7 cfu * / ml) inoculum; L = low (10^3 cfu / ml) inoculum)*

Compound	*S. aureus* [a]	*S. aureus* [b]	*E. coli*	*Pseudomonas aeruginosa*	*Clostridium perfringens*
2b H	> 257	0.51	> 257	> 257	
L	8.23	0.51	32.9	> 257	16.45
2c H	114	7.38	> 230	> 230	
L	29.5	3.69	> 230	> 230	29.5
3a L	0.15	0.15	0.32	0.15	0.15
3b H	202	1.61	50.1	1.61	
L	6.46	0.40	1.61	0.40	3.23
3c H	> 185	2.96	23.7	1.48	
L	91.8	1.48	5.93	0.74	11.85
4b H	> 231	1.85	> 231	> 231	
L	14.7	0.46	> 231	231	57.2
4c H	104	6.69	> 209	> 209	
L	104	3.34	> 209	209	> 209
4d H	87.2	43.6	> 176	> 176	
L	87.2	11.2	> 176	> 176	176
5b H	> 203	12.9	100	> 203	
L	100	6.48	50.2	100	50.2
5c H	> 186	11.9	> 186	186	
L	> 186	11.9	> 186	186	> 186
6 H	336	0.16	43	0.67	
L	5.37	0.16	21.5	0.35	21.5
7 H	> 294	0.59	2.35	2.35	
L	72.9	0.30	0.31	1.17	4.70

[a] β-Lactamase producing.

[b] Non-β-lactamase producing.



2	m	n	R
a	0	–	H
b	1	7	Boc
c	1	11	Boc

1

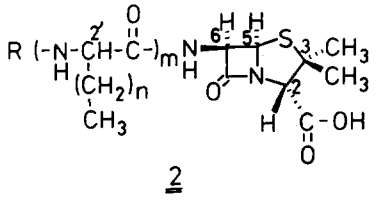
1	n	m
a	7	1
b	11	1
c	7	2

3	n	m	R
a		0	H
b	7	1	Boc
c	11	1	Boc

4	m	n	R
a	0	–	H
b	1	7	Boc
c	1	11	Boc
d	2	7	Boc

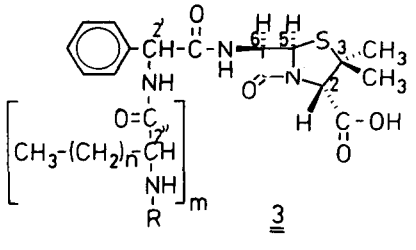
5	m	n	R
a	0	–	H
b	1	7	Boc
c	1	11	Boc

Scheme 1.



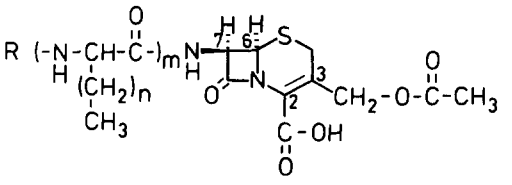
2

Scheme 2.



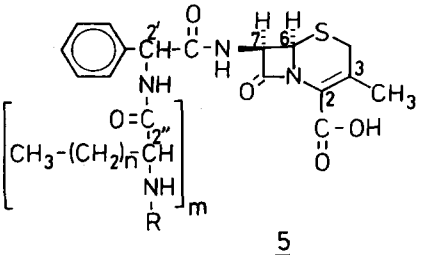
3

Scheme 3.



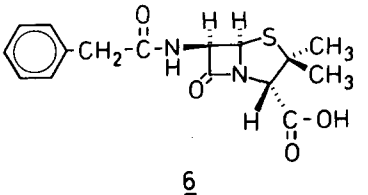
4

Scheme 4.



5

Scheme 5.



6

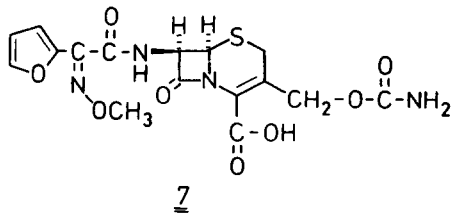
Scheme 6.

TABLE 3

Comparison of in vitro and in vivo (s.c. and p.o.) activity of β-lactam antibiotic amide conjugates against S. aureus 663E

Compound	In vitro MIC ($mmol/l \times 10^{-3}$) (10^7 cfu/ml)	In vivo ED_{50}^{a} ($mmol/kg \times 10^{-3}$)	
		s.c.	p.o.
2b	0.51	> 103	> 103
2c	7.38	> 46.1	> 46.1
3a	0.15 [b]	3.22	3.22
3b	1.61	10.0	> 80.8
3c	2.96	5.33	–
4b	1.85	> 46.1	> 46.1
4c	6.69	> 41.8	> 41.8
4d	43.6	> 35.1	> 35.1
5a		23.9	17.9
5b	12.9	> 40.5	> 40.5
5c	11.9	> 37.1	> 37.1
6	0.16	1.34	9.67
7	0.30	8.47	58.8

[a] Dose required to protect 50% of animals from lethal infection.
[b] 10^3 cfu/ml.



7

Scheme 7.

active against *Clostridium perfringens*. The ampicillin conjugate **3b** was more potent than penicillin G (**6**) and equipotent with cefuroxime (**7**).

In vivo antibiotic activity

The conjugates tested in vivo were administered by both subcutaneous (s.c.) and oral (p.o.) routes to mice that had been previously infected with a non-β-lactamase producing strain of *S. aureus*. An ED_{50} value was obtained for compounds **2b, c, 3b, c, 4b–d, 5b** and **c** and the antibiotics, penicillin G (**6**), ampicillin (**3a**), cephalexin (**5a**) and cefuroxime (**7**) (Table 3).

Only one compound, the ampicillin conjugate **3c**, was active in vivo following subcutaneous administration, with a reduced potency when compared with penicillin G (**6**) or ampicillin (**3a**). None of the other conjugates were orally active. Thus conjugates **3b, c, 5b** and **c** were not effectively cleaved to the parent antibiotics in vivo. The latter results indicate that the β-lactam amide conjugates **2b, c, 3b, c, 4b, d, 5b** and **c** were insufficiently active in their own right to elicit a protective response, as reflected in their modest in vitro potencies.

Conclusions

The penicillin amide derivatives **2b, c, 3b** and **c**, and the cephalosporin conjugates **4b–d, 5b** and **c** were prepared by coupling the appropriate *N*-protected lipidic amino acids and peptides to the parent antibiotics using mixed anhydride methods. All amide conjugates exhibited some antibiotic activity in vitro against a non-β-lactamase producing strain of *S. aureus*. However, the compounds were insufficiently potent to protect against the lethal infection by the same organism in vivo, following either subcutaneous or oral administration. The reduced activity of conjugates compared with that of the parent antibiotics suggests, as expected, that the amide linkage between the parent compound and the lipidic moiety is biologically stable.

The purpose was to conjugate drugs to our novel lipophilic systems to enhance delivery across natural membrane barriers and enhance metabolic stability of the parent antibiotic. The experiments described our first attempts with antibiotic conjugates. We intend to study lipophilic antibiotic conjugates in more detail, in particular to establish the physical structure of the conjugates, the pharmacokinetic profile for the drug conjugates and the rational design of better antibiotic procedures.

Acknowledgements

The authors would like to express their thanks to Mr C.H. James for running the NMR spectra and Mr W. Baldeo for the microanalyses. This work was supported by Glaxo Group Research Ltd, U.K. (Grant No. ESADO56).

References

Abraham, E.P. and Newton, G.G.F., The structure of cephalosporine C. *Biochem. J.*, 79 (1961) 377–393.

Bamberg, P., Ekström, B. and Sjöberg, B., Semisynthetic penicillins, VII. Use of phenacyl 6-aminopenicillinates in penicillin synthesis. *Acta Chem. Scand.*, 21 (1967) 2210–2214.

Batchelor, F.R., Doyle, F.P., Nayler, J.H.C. and Rolinson, G.N., Synthesis of penicillin: 6-amino-penicillanic acid in penicillin fermentations. *Nature*, 183 (1959) 257–258.

Doyle, F.P., Fosker, G.R., Nayler, J.H.C. and Smith, H., Derivatives of 6-aminopenicillanic acid, I. α-aminobenzylpenicillin and some related compounds. *J. Chem. Soc.*, (1962) 1440–1444.

Gibbons, W.A., Hughes, R.A., Charalambous, M., Christodoulou, M., Szeto, A., Aulabaugh, A.E., Mascagni, P. and Toth, I., Lipidic peptides, I. Synthesis, resolution and structural elucidation of lipidic amino acids and their homo- and hetero-oligomers. *Liebigs Ann. Chem.* (1990) 1175–1183.

Kirby, W.M.M. and Kind, A.C., Clinical pharmacology of

ampicillin and hetacillin. *Ann. N.Y. Acad Sci.*, 145 (1967) 291–297.

Leanza, W.J., Christensen, B.G., Rogers, E.F. and Patchett, A.A., Synthesis of guanidino-substituted penicillins and cephalosporins. *Nature*, 207 (1965) 1395–1396.

Loder, B., Newton, G.G.F. and Abraham, E.P., The cephalosporin C nucleus (7-aminocephalosporanic acid) and some of its derivatives. *Biochem. J.*, 79 (1961) 408–416.

Perron, Y.G., Minor, W.F., Holdrege, C.T., Gottstein, W.J., Godfrey, J.C., Crast, L.B., Babel, R.B. and Cheney, L.C., Derivatives of 6-aminopenicillanic acid, I. Partially synthetic penicillins prepared from α-aryloxyalkanoic acids. *J. Am. Chem. Soc.*, 82 (1960) 3934–3938.

Ryan, C.W., Simon, R.L. and Van Heyningen, E.M., Chemistry of cephalosporin antibiotics, XIII. Desacetoxycephalosporins. The synthesis of cephalexin and some analogs. *J. Med. Chem.*, 12 (1969) 310–313.

Ryan, D.M., O'Callaghan, C.H. and Muggleton, P.W., Cefuroxime, a new cephalosporin antibiotic: activity in vivo. *Antimicrob. Agents Chemother.*, 9 (1976) 520–525.

Spencer, J.L., Flynn, E.H., Roeske, R.W., Siu, F.Y. and Chauvette, R.R., Chemistry of cephalosporin antibiotics, VII. Synthesis of cephaloglycin and some homologs. *J. Med. Chem.*, 9 (1966) 746.