Lipidic Peptides. V: Penicillin and Cephalosporin Acid Conjugates with Increased Lipophilic Character

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Abstract □ Lipophilic, double-ester derivatives of β-lactam antibiotics with methylene, ethylene, and propylene spacers were prepared by crown-ether-assisted coupling of halogenoalkyl esters of 2-(*tert*-butoxycarbonylamino)decanoic acid to either penicillin G or cefuroxime. The hydroxyethyl ester of penicillin G and the *tert*-butoxypropyl ester of cefuroxime were also prepared. The lipophilic, double-ester conjugates, the hydroxyethyl ester of penicillin G, and the *tert*-butoxypropyl ester of cefuroxime showed weak or no antibiotic activity in vitro, as expected. The lipophilic penicillin G conjugates and the *tert*-butoxypropyl ester of cefuroxime were active in vivo against a nonpenicillinase-producing strain of *Staphylococcus aureus* after subcutaneous administration. The penicillin G double ester with propylene spacer and the *tert*-butoxypropyl ester of cefuroxime were inactive in vitro, a fact indicating that both compounds were hydrolyzed in vivo, as desired. After oral administration, the lipophilic, double-ester conjugate of penicillin G with methylene spacer and the *tert*-butoxypropyl ester of cefuroxime were active.

Fatty amino acids are α-amino acids with a long, linear or branched alkyl side chain. A number of uses can be envisaged that exploit the amphipathic nature of these compounds and their oligomers.[1] Of particular interest is the use of fatty amino acids and oligomers as conjugating units for biologically active compounds. The conjugates formed would possess a degree of lipidlike or membranelike character due to the alkyl side chains of the fatty compounds. This feature will enhance the passage of poorly absorbed drugs across biological membranes to reach their site of action.

Simple esters of the β-lactam antibiotics are too stable in vivo to be used as prodrugs.[2,3] An intensive research effort to find a derivative of the carboxylic acid function of the β-lactam antibiotics that would improve absorption, yet is sufficiently labile under biological conditions, led to the development of the acyloxymethyl esters of penicillin G[4] and ampicillin.[5] The double-ester linkage common to the antibiotic prodrugs, in which the two ester groups are separated by a methylene spacer, is enzymatically labile. The susceptibility of the methylene-bridged double esters to enzyme-initiated hydrolysis renders them ideal for the bioreversible attachment of lipidic amino acids and peptides to β-lactam antibiotics.

There are several possibilities for conjugation of lipidic amino acids and peptides to the β-lactam antibiotics.[6] We report an investigation of the ability of carboxyl-conjugating lipidic amino acids to improve the oral absorption of β-lactam antibiotics.

Experimental Section

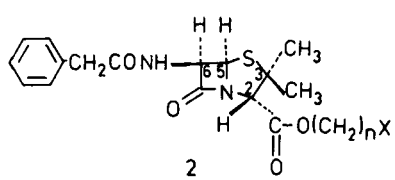
Double-Ester Prodrugs with Methylene Spacer—The chloromethyl ester **1b** was prepared by reacting the potassium salt of the N-*tert*-butoxycarbonyl (Boc)-protected lipidic amino acid **1a**[1] with iodochloromethane in the presence of the macrocyclic ether 18-crown-6. When a dibromomethane was used, the desired bromomethyl ester could not be obtained. The major product of the reaction was the diester **1c**, even when a 10-fold molar excess of dibromomethane was used. The double-ester derivative **2c** was prepared by reacting the ester **1b** with the crown-ether complex of the sodium salt of **2a**. The synthesis of the cefuroxime derivative **3b** was identical to that of the conjugate **2c**, from ester **1b** and the crown-ether complex of the potassium salt of **3a**.

$(CH_3)_3COOCNH[CH_3(CH_2)_7]CHCOO(CH_2)_nX$

1

1	n	X
a	0	H
b	1	Cl
c	1	$OOCCH[(CH_2)_7CH_3]NHCOOC(CH_3)_3$
d	2	Br
e	3	Cl

Double Esters with Ethylene and Propylene Spacers—Another approach in preparing labile esters of the β-lactam antibiotic carboxylic acid group is exemplified by the methicillin aminoethyl ester derivative,[6] in which the ester linkage is believed to be chemically, rather than enzymatically, unstable. Similar behavior might be expected for analogues **2d**, **2e**, **3c**, and **3d**, in which the nitrogen is



2

2	n	X
a	0	H
b	1	$OCOCH_3$
c	1	$OOCCH[(CH_2)_7CH_3]NHCOOC(CH_3)_3$
d	2	$OOCCH[(CH_2)_7CH_3]NHCOOC(CH_3)_3$
e	3	$OOCCH[(CH_2)_7CH_3]NHCOOC(CH_3)_3$
f	2	Br
g	2	OH

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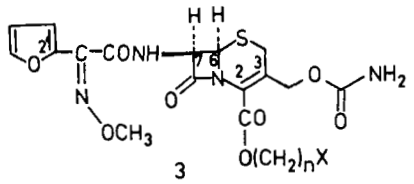

3

3	n	X
a	0	H
b	1	$OOCCH[(CH_2)_7CH_3]NHCOOC(CH_3)_3$
c	2	$OOCCH[(CH_2)_7CH_3]NHCOOC(CH_3)_3$
d	3	$OOCCH[(CH_2)_7CH_3]NHCOOC(CH_3)_3$
e	3	$OC(CH_3)_3$

replaced by an oxygen atom. Conjugates **2d**, **2e**, **3c**, and **3d** were prepared with an ethylene or propylene bridge between the parent compound and the lipidic delivery system. The bromoethyl ester **1d** and chloropropyl ester **1e** were prepared from acid **1a** and 1,2-dibromoethane or 1-chloro-3-iodopropane, respectively, by using the crown-ether-assisted method. Double-ester ampicillin conjugates **2d** and **2e** and cefuroxime conjugates **3c** and **3d** were prepared by reacting the potassium–crown-ether complex of ampicillin (**2a**) and cefuroxime (**3a**) with halogeno esters **1d** and **1e**. An interesting side reaction took place during the coupling of **3a** and the chloropropyl ester **1e**. In addition to the desired double-ester conjugate **3d**, the *tert*-butyl ether **3e** was formed. To compare the alternative synthetic strategy for the double-ester conjugates, ester **2f** was prepared from 1,2-dibromoethane and penicillin G (**2a**). The synthesis of the conjugate **2d** was completed by reacting ester **2f** with acid **1a**. Esterase attack of conjugate **2d** would yield the hydroxyethyl ester **2g** as a major metabolite. Compound **2g** was synthesized from 2-bromoethanol and penicillin G (**2a**) to assess its biological activity.

Chemistry—IR spectra were recorded with a Perkin-Elmer 841 spectrophotometer. ^1H 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 parts per million downfield from internal tetramethylsilane. Mass spectra were run on a VG Analytical ZAB-SE instrument, with fast-atom bombardment ionization. Reaction progress was monitored by thin-layer chromatography on silica gel (Kieselgel PF_{254}), with dichloromethane:methanol (10:1) as the mobile phase. Purification was by flash chromatography through Kieselgel G (toluene:methanol, 10:1). Solvents were evaporated under reduced pressure with a rotary evaporator. Melting points are not given for enantiomers. The experimental data are summarized in Table I.

Method A—*Chloromethyl 2-(tert-butoxycarbonylamino)decanoate (1b)*—Compound **1a** (1.0 g, 3.48 mmol) was stirred in ethanol (5 mL) and water (5 mL). KOH (195 mg, 3.48 mmol) and 18-crown-6 (952 mg, 3.60 mmol) were added, and the reaction mixture was stirred for 30 min at room temperature. The solvents were evaporated, and the residue was dried under reduced pressure. The crown-ether complex was dissolved in dry dimethylformamide (5 mL), chloroiodomethane (2.0 g, 11.34 mmol) was added, and the solution was stirred for 24 h at room temperature. The reaction mixture was diluted with ethyl acetate (50 mL) and washed successively with brine (50 mL), 3% aqueous $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer, after drying with Na_2SO_4, was evaporated, and the residue was purified by flash chromatography; ^1H NMR ($CDCl_3$): δ 5.82, 5.62 (2H, q, OCH_2Cl), 4.95 (1H, d, NH), 4.35 (1H, m, α-CH), 1.85, 1.65 (2H, m, CH_2), 1.42 [9H, s, $C(CH_3)_3$], 1.28 (12H, m, 6 CH_2), 0.90 (3H, t, CH_3); MS: *m/z* (%) 358 $[M + Na]^+$ (100), 332 (10), 302 (29), 280 (13), 236 (33), 186 (25), 173 (30), 142 (40), 81 (30), 57 (91), 41 (33).

Method B—*2'-[(tert-Butoxycarbonylamino)decanoyloxymethyl] (2'SR,2S,5R,6R)-3,3-Dimethyl-7-oxo-6-phenylacetimido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2c)*—Penicillin G (**2a**) potassium salt (500 mg, 1.342 mmol) was reacted with **1b** (450 mg, 1.3425 mmol) as described for method A; ^1H NMR ($CDCl_3$): δ 7.32 (5H, m, aromatic H), 6.05 (1H, d, NH), 5.81 (2H, ab, OCH_2O), 5.63 (1H, 2d, C_6–H), 5.47 (1H, d, C_5–H), 4.85 (1H, d, NH), 4.40 (1H, s, C_2–H), 3.62 (2H, s, benzyl CH_2), 1.76 (2H, m, β-CH_2), 1.48, 1.45 (6H, 2s, 2 CH_3), 1.43 [9H, s, $C(CH_3)_3$], 1.30 (12H, m, 6 CH_2), 0.86 (3H, t, CH_3); MS: *m/z* (%) 656 $[M + Na]^+$ (54), 600 (20), 482 (4), 422 (5), 403 (10), 332 (15), 254 (7), 232 (20), 198 (7), 186 (15), 173 (10), 160 (20), 142 (34), 114 (8), 91 (28), 57 (100), 41 (28).

2'-[(tert-Butoxycarbonylamino)decanoyloxyethyl] (2'SR,2S,5R,6R)-3,3-Dimethyl-7-oxo-6-phenylacetimido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2d)—^1H NMR ($CDCl_3$): δ 7.34 (5H, m, aromatic H), 6.08 (1H, d, NH), 5.65 (1H, m, C_6–H), 5.48 (1H, m, C_5–H), 4.93 (1H, m, NH), 4.42 (1H, s, C_2–H), 4.38 (4H, m, 2 CH_2), 3.50 (2H, s, benzyl CH_2), 1.85 (2H, m, β-CH_2), 1.49, 1.46 (6H, s, 2 CH_3), 1.43 [9H, s, $C(CH_3)_3$], 1.27 (12H, m, 6 CH_2), 0.85 (3H, t, CH_3); MS: *m/z* (%) 670 $[M + Na]^+$ (67), 625 (13), 624 (35), 567 (6), 495 (5), 455 (23), 417 (21), 395 (13), 258 (12), 240 (10), 186 (33), 142 (100), 91 (32), 73 (41).

2'-[(tert-Butoxycarbonylamino)decanoyloxypropyl] (2'SR,2S,5R,6R)-3,3-Dimethyl-7-oxo-6-phenylacetimido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2e)—^1H NMR ($CDCl_3$): δ 7.30 (5H, m, aromatic H), 6.05 (1H, d, NH), 5.65 (1H, m, C_6–H), 5.50 (1H, d, C_5–H), 4.95 (1H, m, NH), 4.35 (1H, s, C_2–H), 4.17 (5H, m, $COOCH_2$, OCH_2, α-CH), 3.65 (2H, m, benzyl CH_2), 2.05 (2H, 2t, CH_2), 1.75 (2H, m, β-CH_2), 1.55 (6H, s, 2 CH_3), 1.40 [9H, s, $C(CH_3)_3$], 1.25 (12H, m, 6 CH_2), 0.90 (3H, t, CH_3); MS: *m/z* (%) 684 $[M + Na]^+$ (100), 628 (17), 584 (6), 562 (4), 534 (4), 509 (4), 487 (9), 431 (40), 409 (16), 387 (17), 366 (3), 272 (5), 218 (6), 186 (5), 142 (9), 92 (6).

Hydroxyethyl (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-phenylacetimido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2g)—^1H NMR ($CDCl_3$): δ 8.00 (1H, s, OH), 7.75 (5H, m, aromatic H), 6.15 (1H, d, NH), 5.65 (1H, m, C_5–H), 5.55 (1H, d, C_6–H), 4.41 (1H, s, C_2–H), 4.30 (2H, m, $COOCH_2$), 3.90, 3.85 (2H, m, CH_2OH), 3.50 (2H, m, benzyl CH_2), 1.48, 1.43 (6H, s, 2 CH_3); MS: *m/z* (%) 379 $[M + H]^+$ (10), 204 (100), 160 (10), 132 (12), 114 (25), 91 (81), 73 (21).

2'-(tert-Butoxycarbonylamino)decanoyloxymethyl (2'SR,6R,7R)-3-Carbamoyloxymethyl-7-[furan-2-yl(methoxyimino)acetamido]-8-

Table I—Summary of Experimental Data

Product (Starting Compounds)	Yield, %[a]	R_f[b]	Analysis[c]
2c (2a, 1b)	27	0.40	$C_{32}H_{47}N_3O_8S$ (633.8). Calc.: C, 60.64; H, 7.47; N, 6.33. Found: C, 60.36; H, 7.52; N, 6.53.
2d (2a, 1d)	41.3	0.42	$C_{33}H_{49}N_3O_8S$ (647.8). Calc.: C, 61.18; H, 7.62; N, 6.49. Found: C, 60.90; H, 7.79; N, 6.37.
2e (2a, 1e)	57.3	0.42	$C_{54}H_{51}N_3O_8S$ (661.8). Calc.: C, 61.70; H, 7.77; N, 6.35. Found: C, 61.70; H, 7.84; N, 6.21.
2g (2a, 2-bromoethanol)	41.3	0.39	$C_{18}H_{22}N_2O_5S$ (378.4). Calc.: C, 57.13; H, 5.59; N, 7.40. Found: C, 57.00; H, 6.65; N, 7.18.
1b (1a, chloroiodomethane)	73.5	0.98	$C_{16}H_{30}ClNO_4$ (335.8). Calc.: C, 57.22; H, 9.00; N, 4.17. Found: C, 57.01; H, 9.20; N, 3.99.
3b (3a, 1b)	42	0.45	$C_{32}H_{45}N_5O_{12}S$ (723.8). Calc.: C, 53.10; H, 6.27; N, 9.68. Found: C, 52.99; H, 6.37; N, 9.39.
3c (3a, 1d)	36.9	0.46	$C_{33}H_{47}N_5O_{12}S$ (737.8). Calc.: C, 53.72; H, 6.42; N, 9.49. Found: C, 53.53; H, 6.57; N, 9.19.
3e (3a, 1e)	26.5	0.52	$C_{23}H_{30}N_4O_8S$ (522.5). Calc.: C, 52.87; H, 5.79; N, 10.72. Found: C, 52.69; H, 5.81; N, 10.60.

[a] All products were synthesized by method B, except for **1b**, which was synthesized by method A. All products were oily solids, except for **2g** and **1b**, which were oils. [b] Retardation factor. [c] Values in parentheses are formula weights.

oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (3b)—^{1}H NMR ($CDCl_3$): δ 7.40 (1H, m, NH), 6.90 (1H, m, $C_{5'}$–H), 6.48 (2H, m, $C_{3'}$–H, $C_{4'}$–H), 5.92, 5.10 (2H, ab, m, OCH_2O), 5.81 (1H, m, C_7–H), 5.10 (1H, m, C_6–H), 5.35, 4.60 (2H, ab, m, OCH_2), 4.95 (1H, m, NH), 4.28 (1H, m, α-CH), 4.10 (3H, s, OCH_3), 3.82, 3.48 (2H, ab, $2C_4$–H), 1.85, 1.62 (2H, m, 2CH), 1.25 (12H, m, $6CH_2$), 0.85 (3H, t, CH_3); MS: *m/z* (%) 746 $[M + Na]^+$ (11), 641 (13), 332 (44), 298 (7), 232 (100), 173 (10), 119 (12), 92 (10), 57 (45).

2'-(tert-Butoxycarbonylamino)decanoyloxyethyl (2'SR,6R,7R)-3-Carbamoyloxymethyl-7-[furan-2-yl(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (3c)—^{1}H NMR ($CDCl_3$): δ 7.45 (1H, m, NH), 6.87 (1H, m, $C_{5'}$–H), 6.46 (2H, m, $C_{3'}$–H, $C_{4'}$–H), 5.76, 5.37 (2H, m, C_6–H, C_7–H), 5.10 (1H, m, NH), 5.95, 5.05 (2H, ab, m, OCH_2), 5.10 (1H, m, NH), 4.35, 4.45 (4H, m, OCH_2CH_2O), 4.30 (1H, m, α-CH), 4.05 (3H, s, OCH_3), 3.65, 3.40 (2H, ab, $2C_4$–H), 1.78, 1.57 (2H, m, 2CH), 1.41 [9H, s, $C(CH_3)_3$], 1.25 (12H, m, 6 CH_2), 0.85 (3H, t, CH_3); MS: *m/z* (%) 760 $[M + Na]^+$ (23), 704 (3), 413 (2), 354 (3), 298 (2), 258 (7), 232 (4), 186 (8), 142 (36), 73 (41), 57 (100).

tert-Butoxypropyl (6R,7R)-3-Carbamoyloxymethyl-7-[furan-2-yl(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (3e)—^{1}H NMR ($CDCl_3$): δ 7.20 (1H, d, NH), 6.89 (1H, d, $C_{5'}$–H), 6.46 (2H, 2d, $C_{4'}$–H, $C_{3'}$–H), 5.98 (1H, dd, 7H), 5.19, 5.12, 4.87, 4.82 (2H, ab, OCH_2), 5.09 (1H, d, C_6–H), 4.70 (2H, m, NH_2), 4.52, 4.38 (2H, 2m, 2OCH), 4.09 (3H, s, OCH_3), 3.66 (2H, t, OCH_2), 3.60, 3.51 (2H, ab, $2C_4$–H), 2.17 (2H, m, CH_2), 1.60 [9H, s, $C(CH_3)_3$]; MS: *m/z* (%) 523 $[M + H]^+$ (100), 476 (10), 413 (20), 386 (36), 349 (20), 326 (68), 199 (70), 154 (30), 136 (30), 92 (45), 77 (15), 63 (20).

Spectral data and analysis of intermediates **1c–1e**, **2f**, and **3d** were as expected.

Measurement of In Vitro Activity—Minimum inhibitory concentrations (MICs) were determined by incorporation of compounds into Iso-Sensitest agar (Oxoid Ltd.). Final levels in the medium were in the range 0.33–0.2 × 10^{-3} mmol/L. Aerobic test organisms were applied to the agar with a 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 were 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, Beckton Dickinson) as appropriate. MICs were recorded as the lowest concentration completely inhibiting visible bacterial growth (Table II).

Experimental Chemotherapy—Protection tests in mice were performed with methods similar to those in the literature[7]; a nonpenicillinase-producing strain of *Staphylococcus aureus* (strain 663E) was used. Female CD1 mice (18–20 g) were challenged intraperitoneally with ~10 times the 50% lethal dose (1.25 × 10^6 cfu/mouse) of bacteria. The bacteria were 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 sulfoxide (final concentration, 10%); this was followed by serial fourfold dilutions in 0.5% sodium carboxymethyl cellulose containing 10% dimethyl sulfoxide. Dose levels generally ranged from 1.0 × 10^{-3} to 66 × 10^{-3} mmol/kg. Five mice were used for each compound concentration, and doses (0.2 mL) were administered either subcutaneously (sc) or orally (po) at 1 and 5 h postchallenge. The median dose required to protect 50% of animals from lethal infection (ED_{50}; mmol/kg/dose) was calculated by logit transformation from the number of animals surviving at each dose level on day 5 (Table III).

Table II—MICs of Conjugates of β-Lactam Antibiotics against Various Bacteria.

Compound and Inoculum[a]	MIC × 10^{-3}, mmol/L				
	SA 1033E[b]	SA 663E[c]	EC 1852e[d]	PA 2033E[e]	CF 2045E[f]
2c					
H	>197	0.2	>197	3.1	
L	197	0.2	>197	0.7	197
2d					
H	>192	3.1	>192	24	
L	192	0.38	>192	3.1	24
2e					
H	>188	>188	>188	>188	
L	>188	>188	>188	>188	93
2g					
H	>330	21	>330	164	
L	164	10	>330	42	164
3b					
H	>172	85	85	>172	
L	>172	>172	22	>172	22
3c					
H	>170	>170	>170	>170	
L	>170	>170	>170	>170	42
3e					
H	>239	>239	118	>239	
L	>239	239	118	>239	59

[a] H, high (10^7 cfu/mL); L, low (10^3 cfu/mL). [b] β-Lactamase-producing *S. aureus*. [c] Non-β-lactamase-producing *S. aureus*. [d] *Escherichia coli*. [e] *P. aeruginosa*. [f] *C. perfringens*.

Results and Discussion

In Vitro and In Vivo Activities—The in vitro activities of the conjugates **2c–2e**, **2g**, **3b**, **3c**, and **3e** were determined against a range of organisms (Table II). Most of the conjugates were inactive or only weakly active against all organisms, as expected. However, the conjugates **2c** and **2d**, with methylene and ethylene ester spacers, respectively, showed appreciable activity against *Pseudomonas aeruginosa* and a nonpenicillinase-producing strain of *S. aureus*. The results indicate that conjugates **2c** and **2d** may have been partially hydrolyzed under the in vitro assay conditions to give the parent antibiotic, penicillin G (**2a**), and the hydroxyethyl ester **2g**, respectively. The latter derivative showed weak activity against *S. aureus* 663E, *P. aeruginosa*, and *Clostridium perfringens*.

The antibiotic activities of the lipidic amino acid double-ester conjugates **2c–2e**, the hydroxyethyl ester **2g**, and the derivative **3e** were determined in vivo (Table III). All compounds, except the hydroxyethyl ester **2g**, were active against *S. aureus* 663E after sc administration in the mouse. Two esters, the penicillin double ester with a propylene bridge (**2e**) and the ether (**3e**), were inactive against the same organism in vitro, a result suggesting that both **2e** and **3e** may have been hydrolyzed in vivo, as desired. After po administration, the double-ester conjugate of penicillin G with a methylene spacer between the two ester groups (**2c**) and the *tert*-butyl ether (**3e**) were active. Indeed, the antibiotic activity of conjugate **2c** against *S. aureus* 663E was as good as that of ampicillin. Given that the parent compound penicillin G (**2a**) had little or no oral activity, the result showed that the conjugation of a lipidic amino acid via a bioreversible ester linkage imparted a positive effect on the absorption of a β-lactam antibiotic.

Conclusions

Double-ester derivatives of β-lactam antibiotics with methylene (**2c** and **3b**), ethylene (**2d** and **3c**), and propylene (**2e** and **3d**) spacers were prepared by crown-ether-assisted coupling of a halogenoalkyl ester of 2-(*tert*-butoxycarbonylamino)decanoic acid (**1b**, **1d**, and **1e**) to either penicillin G (**2a**) or cefuroxime (**3a**). The hydroxyethyl ester **2g** and *tert*-butoxypropyl ester **3a** were also prepared. The double-ester conjugates **2c–2e**, **3b**, and

Table III—In Vitro and In Vivo Activities of Conjugates of β-Lactam Antibiotics against *S. aureus* 663E

Compound	In Vitro MIC × 10^{-3}, mmol/L (10^7 cfu/mL)	In Vivo ED_{50} × 10^{-3}, mmol/kg	
		sc	po
2c	0.2	<2.0	<2.0
2d	3.0	2.0	>38
2e	>37	<2.0	>37
2g	21	>66	>66
3e	>48	4.5	15

3c; the hydroxyethyl ester 2g; and the *tert*-butoxypropyl ester 3e showed weak or no antibiotic activity in vitro, as expected. Compounds 2c, 2d, 2e, and 3e were active after sc administration. The double ester 2e and the ether 3e were inactive in vitro, a result indicating that both 2e and 3e acted as prodrugs. After po administration, the double-ester conjugate 2c and the *tert*-butyl ether 3e were active. Indeed, the antibiotic activity of conjugate 2c was as good as that of ampicillin.[6] The results show that the conjugation of a lipidic amino acid via a bioreversible ester linkage imparts a positive effect on the absorption of a β-lactam antibiotic.

References and Notes

1. Gibbons, W. A.; Hughes, R. A.; Charalambous, M.; Christodoulou, M.; Szeto, A.; Aulabaugh, A. E.; Mascagni, P.; Toth, I. *Liebigs Ann. Chem.* **1990**, 1175–1183.
2. Agersborg, H. P. K.; Batchelor, A.; Cambridge, G. W.; Rule, A. W. *Br. J. Pharmacol.* **1966**, *26*, 649–655.
3. Holysz, R. P.; Stavely, H. E. *J. Am. Chem. Soc.* **1950**, *72*, 4760–4763.
4. Ferres, H. W. *Chem. Ind.* **1980**, 435–450.
5. Daehne, W. V.; Frederiksen, E.; Gundersen, W. O.; Lund, F.; Morch, P.; Petersen, H. J.; Roholt, K.; Tybring, L.; Godtfredsen, W. O. *J. Med. Chem.* **1970**, *13*, 607–612.
6. Toth, I.; Hughes, R. A.; Ward, P.; Baldwin, M. A.; Welham, A. M.; McColm, A. M.; Cox, D. M.; Gibbons, W. A. *Int. J. Pharm.* **1991**, *73*, 259–266.
7. Ryan, D. M.; O'Callaghan, C. H.; Muggleton, P. W. *Antimicrob. Agents Chemother.* **1976**, *9*, 520–525.

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BOOK REVIEWS

Biotransformations: A Survey of the Biotransformations of Drugs and Chemicals in Animals. Volume 2. Edited by D. R. Hawkins. Royal Society of Chemistry; Cambridge, UK. 1989. xix + 496 pp. 19 × 25 cm. ISBN 0-85186-167-9. $153.00.

An understanding of the biological fate of xenobiotics is an essential element in defining their toxicological and/or pharmacological profiles. Since opportunities for studying comparative metabolism in humans are limited, it is important that information obtained from experiments conducted in animals be applied appropriately to predict biotransformation pathways in humans. A knowledge of pharmacokinetics based on biotransformation profile is often indispensable in assessing the pharmacological/toxicological properties of a new investigational drug. Because the toxicity or pharmacological profile of a drug or chemical is often dependent on a particular functional group rather than the whole structure, a knowledge of biotransformations in vertebrates based on chemical entities of a variety of chemicals, such as pharmaceuticals, agrochemicals, food additives, and naturally occurring or synthetic chemicals, could be an important tool in designing the toxicity studies of new chemicals.

The second volume is identical in format to the first and is an excellent source of information on xenobiotic biotransformations based on key functional groups and chemical entities. To aid the reader in understanding the relationship between a functional group and overall metabolism, an overview on key functional groups is provided, followed by a chapter that focuses on unique biotransformation pathways, species differences, and possible mechanisms of toxicity. This chapter is particularly useful in predicting unexpected metabolites which occur (and their toxicological/pharmacological profiles) when heterocyclic rings are cleaved or new ring systems are formed by cyclization of bifunctional intermediates.

Abstracts depicting biotransformation pathways are arranged according to compound classes, although there may be cases where allocation to a particular class is somewhat subjective. The classes of compounds have been carefully selected to include most, if not all, groups of compounds. The biotransformation pathways are consistent with the current literature. No information is provided with regard to species and/or sex differences in metabolism of a particular chemcial or chemicals with similar structures. In each abstract, attempts have been made to provide key information such as qualitative and quantitative (percentage of administered dose and route differences in excretion) aspects of the metabolites. Useful information on radiolabeling of positions is presented. Analytical procedures used for separation and isolation of metabolites are briefly discussed but are not followed by appropriate references.

This book should serve as a valuable reference for investigators and students requiring information concerning compound-specific biotransformations and could become a valuable tool for predicting biotransformation of new compounds with similar structural features.

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Current Books

Pulsed-Field Gel Electrophoresis. Protocols, Methods, and Theories. Edited by M. Burmeister and L. Ulanovsky. Humana: Totowa, N.J. 1992. 488 pp. 24 × 16 cm. ISBN 0-89603-229-9. $69.50.

Clinical Primer of Psychopharmacology. A Practical Guide. By D. M. Perodsky and C. J. S. Cohen. McGraw Hill, New York. 1992. 129 pp. 21 × 14 cm. ISBN 0-07-105388-3. $19.95.

Serological Cancer Markers. Edited by Stewart Sell. Humana: Totowa, NJ. 1992. 552 pp. 23 × 16 cm. ISBN 0-89603-209-4. $99.50.

Plant Cell and Tissue Culture in Liquid Systems. By G. F. Payne, V. Bringi, C. L. Prince, and M. L. Schuler. Oxford University Press: New York. 1992. 346 pp. 24 × 18 cm. ISBN 3-446-15830-8. $88.00.

The Alkaloids. Chemistry and Pharmacology. Volume 41. Edited by A. Brossi and G. A. Cordell. Academic Press: New York. 1992. 252 pp. 25 × 16 cm. ISBN 0-12-469541-8. $85.00.

Treatise on Controlled Drug Delivery: Fundamentals, Optimization, Applications. Edited by A. Kydonieus. Marcel Dekker: New York. 1991. 568 pp. 26 × 18 cm. ISBN 0-8247-8519-3. $135.00.

Electrophysiology and Pharmacology of the Heart: A Clinical Guide. Edited by K. H. Dangman and D. S. Miura. Marcel Dekker: New York. 1991. 784 pp. 26 × 18 cm. ISBN 0-8247-8449-9. $175.00.

Beta-Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology. Edited by R. R. Ruffolo, Jr. Karger: Basel, Switzerland. 1991. 240 pp. 24 × 17 cm. ISBN 3-8055-5502-4. $196.00.

Compendium of Organic Synthetic Methods. By Michael B. Smith. Wiley: New York. 1992. 547 pp. 25 × 17 cm. ISBN 0-471-60713-4. No price given.