Structure-Activity Relationships in Cephalosporins Prepared from Penicillins. 2. Analogues of Cephalexin Substituted in the 3-Methyl Group

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A previously outlined general procedure for preparing various 3-substituted cephalosporins from the penicillin nucleus has been used, with modifications where required, to prepare a series of analogues of cephalexin with various substituents in the 3-methyl group. The 3-substituents most conducive to broad-spectrum antibacterial activity were 3-pyridylmethyl and *m*- or *p*-carboxybenzyl. The compounds were only poorly absorbed by the oral route in mice, but the 3-(carboxybenzyl) compounds gave more prolonged useful serum levels than the usual cephalosporins.

We have previously reported[1,2] a multistep procedure for converting the penicillin nucleus into cephalosporins. In the preceding paper[3] we described various 7β-acylamino derivatives of 3-benzyl- and 3-(3-pyridylmethyl)ceph-3-em-4-carboxylic acids. In both the benzyl and the 3-pyridylmethyl series the D-phenylglycyl derivative showed relatively good antibacterial activity against both gram-positive and gram-negative bacteria, so we decided to employ this acyl group as our derivative of choice for evaluating the potential of other cephem nuclei. Hence in this paper we report a series of analogues of cephalexin (**12**, X = H) with various substituents in the 3-methyl group.

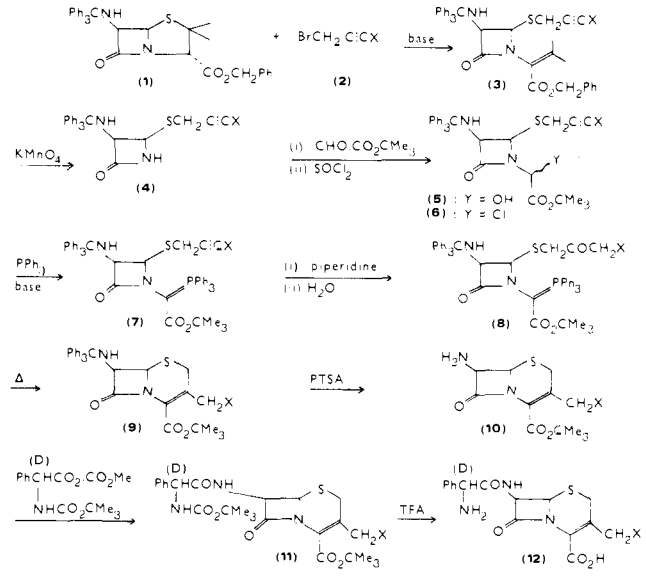
Chemistry. The various cephalexin analogues (**12**) were prepared as previously described[3] for the 3-benzyl and 3-(3-pyridylmethyl) analogues, i.e., by treating the appropriate *tert*-butyl 7β-aminoceph-3-em-4-carboxylate (**10**) with a mixed anhydride derived from *N*-(*tert*-butoxycarbonyl)-D-phenylglycine and then removing both acid-labile protecting groups by means of TFA. In those cases where the final cephalosporin contained a second carboxyl group as part of the 3-substituent, this function was also protected during the earlier part of the synthesis as a *tert*-butyl or *p*-methoxybenzyl ester and the final TFA treatment removed three protecting groups.

The requisite *tert*-butyl 3-substituted 7β-aminoceph-3-em-4-carboxylates (**10**) were synthesized from the penicillin derivative, benzyl 6β-(triphenylmethylamino)-penicillanate (**1**). In most cases the synthesis involved a precisely similar reaction sequence (Scheme I) to that previously used[1,2] to prepare *tert*-butyl 7β-amino-3-benzylceph-3-em-4-carboxylate (**10**, X = Ph).

The only case encountered of a 3-substituted prop-2-ynyl bromide (**2**) which failed to give a 1,2-secopenicillanate **3** on reaction with benzyl 6β-tritylaminopenicillanate (**1**) in the presence of base was the methoxycarbonyl compound **2** (X = CO_2Me). Hence, in order to synthesize **25** we prepared the unsubstituted prop-2-ynyl sulfide **7** (X = H) according to Scheme I, treated it with butyllithium followed by carbon dioxide to give the crude acid **7** (X = CO_2H), esterified the latter with diazomethane, and then again proceeded according to Scheme I.

A modified sequence was also used to prepare the 1-methyl-2-pyrrolyl derivative **20**. For this purpose the prop-2-ynyl sulfide **3** (X = H) was treated with iodine nitrate to give the iodopropyne **3** (X = I), which was converted according to Scheme I into **7** (X = I). Treatment of the latter with 1-methyl-2-pyrrolylcopper(I) then gave **7** (X = 1-methyl-2-pyrrolyl). The remaining steps then followed Scheme I except that, because of the sensitivity of pyrroles to acids, we used pyridine hydrochloride instead of *p*-toluenesulfonic acid to detritylate **9** (X = 1-methyl-2-pyrrolyl). The route via **7** (X = I) should be capable of extension to other 3-aralkylcephems and would obviate the need to start with a different 3-substituted prop-2-ynyl bromide (**2**) in each case.

Scheme I



In one case the 3-substituent was modified after completion of the cephem ring system and introduction of the protected D-phenylglycine side chain. Thus, treatment of the cephem **45** with *p*-toluenesulfonic acid and isopropenyl acetate removed the tetrahydropyranyl group and replaced it by acetyl to give the 3-(β-acetoxyethyl)ceph-3-em **46**.

Biological Results and Discussion. Table I shows the activity of the new cephalosporins against two gram-positive and five gram-negative bacteria in vitro, corresponding data for two clinically useful compounds with the same D-phenylglycyl side chain (cephalexin and cephaloglycin) being included for comparison.

Staphylococcus aureus Oxford typifies a gram-positive organism which is normally sensitive to β-lactam antibiotics and, as expected, all the compounds in Table I were reasonably active against it. They were rather less active against the β-lactamase-producing *Staphylococcus aureus* Russell, and the MIC figures show that a number of the new cephalosporins are less stable than cephalexin or cephaloglycin toward staphylococcal β-lactamase.

Activity against the gram-negative organisms varied considerably. That of the 3-benzyl compound **13** described in paper 1[3] was not improved by introduction of neutral substituents into the benzene ring (compounds **14** and **15**) but was enhanced by a carboxyl substituent. When this carboxyl group occupied the ortho position (compound **16**) activity was only slightly improved, but the meta and para isomers (**17** and **18**) were about as active as cephalexin, cephaloglycin, or the 3-(3-pyridyl)methyl compound **22** described in paper 1.[3] In the pyridyl series introduction

Table I. Antibacterial Activity of Cephalexin Analogues (12)

Compd	X	Minimum inhibitory concentration, μg/mL[a]						
		S. aureus Oxford	*S. aureus* Russell[b]	*E. coli*	*S. typhi*	*Sh. sonnei*	*K. aerogenes*	*P. mirabilis*
Cephalexin	H	1.25	5	12.5	5	5	5	12.5
Cephaloglycin	OCOMe	1.25	2.5	5	1.25	2.5	5	5
13	Ph	0.1	2.5	25	25	25	25	50
14	C_6H_4-*o*-CF_3	0.5	5	>100	>100	>100	>100	>100
15	C_6H_4-*p*-SO_2NMe_2	0.5	500	25	25	25	50	125
16	C_6H_4-*o*-CO_2H	5	12.5	12.5	50	12.5	5	5
17	C_6H_4-*m*-CO_2H	0.25	1.25	12.5	2.5	25	2.5	2.5
18	C_6H_4-*p*-CO_2H	0.5	2.5	12.5	0.5	5	2.5	1.25
19	2-c-$C_5H_{10}O$	5	5	>50	25	25	10	>50
20	2-C_4H_3N-1-Me	1.25	50	500	50	500	500	500
21	2-C_5H_4N	1.25	12.5	50	12.5	25	>500	50
22	3-C_5H_4N	0.25	2.5	2.5	1.25	2.5	2.5	5
23	3-C_5H_3N-4-Me	0.12	12.5	12.5	5	12.5	12.5	25
24	3-C_5H_3N-5-CO_2H	1.25	12.5	12.5	2.5	12.5	1.25	1.25
25	CO_2Me	0.5	16	31	8	16	63	63
26	CH_2OCOMe	0.5	10	>50	25	50	25	>50

[a] Determined by serial dilution on nutrient agar using an inoculum of 0.001 mL of an undiluted overnight broth culture. MIC values were read after incubation at 37 °C for 18 h. [b] β-Lactamase-producing benzylpenicillin-resistant strain.

Table II. Blood Levels in Mice

Compd	Route	μg/mL following 50 mg/kg dose[a]					
		10 min	20 min	30 min	1 h	2 h	4 h
Cephalexin	po	(23.9)[b]		21.7	8.3	2.5	0.56
Cephaloglycin	po	1.7	2.6	2.0	0.6	0.7	0.07
13	po	0.11	0.18	0.21	0.19	0.34	0.15
16	po	1.1	1.4	2.4	3.2	2.8	3.2
17	po	0.87	1.05	1.8	1.7	1.6	0.55
18	po	<1	<1	1.6	2.4	2.3	1.9
22	po	2.85	1.44	1.25	0.62	0.65	0.10
24	po	0.55	0.85	1.7	1.5	0.6	<0.4
Cephalexin	sc	27.0	20.5	13.3	3.0	0.42	<0.15
Cephaloglycin	sc	37.1	30.2	21.7	3.1	0.20	<0.1
Cephaloridine	sc	32.8	24.4	18.5	5.6	0.71	<0.2
16	sc	63.2	66.0	58.0	49.2	40.6	31.2
17	sc	84.2	64.2	49.6	26.2	13.7	1.6
18	sc	75.2	87.4	68.8	48.0	22.0	9.3
24	sc	70.4	54.0	35.8	12.2	2.0	<0.4
Cephaloglycin	iv	62.4	40.2	11.0	1.5	<0.1	<0.1
Cephaloridine	iv	48.8	23.2	14.3	2.9	0.32	<0.05
16	iv	102	83.6	75.4	64.4	29.1	18.1
18	iv	70.3	48.2	39.2	25.4	4.5	2.4

[a] Groups of five mice were killed at each time interval. Blood was collected from the cut axilla region, heparinized, stored at 4 °C, and subjected on the day of sampling to large plate microbiological assay using *Sarcina lutea* NCTC 8340 with overnight incubation at 30 °C. Standard solutions of the appropriate cephalosporins were prepared in mouse blood. Phosphate buffered saline, pH 7.2, was used as diluent when required. [b] At 15 min.

of a carboxyl group (**24**) did not improve activity further, in contrast to the previously noted effect in the benzyl series. The 3-(2-pyridyl)methyl- (**21**) and 3-(4-methyl-3-pyridyl)methyl- (**23**) cephalosporins were rather less active than **22**, and the compounds with nonbasic heterocyclic substituents (**19** and **20**) were considerably less active. Finally, two compounds structurally related to cephaloglycin, the "reversed ester" **25** and the higher homologue **26**, showed only moderate activity.

In summary, the most active of the new cephalosporins (**17**, **18**, and **22**) were about as active as the cephalosporins currently in clinical use. The breadth of the antibacterial spectrum was also similar to that of the established compounds, normally cephalosporin-resistant gram-negative organisms such as *Pseudomonas aeruginosa*, *Proteus morganii*, and *Enterobacter cloacae* being resistant to the new compounds also.

The distinctive property of cephalexin is that, in contrast to the great majority of cephalosporins, it is well absorbed when given by mouth. Compounds **13**, **16–18**, **22**, and **24** were accordingly administered to groups of mice at 50 mg/kg po and blood levels of the antibiotic were determined by microbiological assay at intervals thereafter. Table II shows that **13** gave only very low levels, while the other compounds gave peak levels of the same order as those given by cephaloglycin. All the peak levels were much lower than that achieved with cephalexin, but levels of the carboxybenzylcephalosporins **16–18** appeared to be relatively well maintained 2 and 4 h after administration.

Blood levels resulting from parenteral administration of the carboxy compounds **16–18** and **24** were also determined, and in all cases the peak levels were found to exceed those of the reference cephalosporins. As in the oral experiments, marked prolongation of significant blood levels was seen with the carboxybenzyl compounds **16–18** but not with the carboxypyridyl compound **24**.

Urine from mice which had received **17** and **18** was subjected to paper strip chromatography using a butanol–ethanol–water (4:1:5) solvent system. In the case of **18**, but not **17**, visualization on agar plates seeded with *Sarcina lutea* revealed a second zone of antibacterial activity due to an unidentified active metabolite. No active metabolite could, however, be detected by similar chromatography of blood samples, so the figures in Table II

Table III. Comparison of Activity of Cephalexin and Compound 18 in Infected Mice

		CD_{50}, mg/kg, total dose			
		Cephalexin		18	
Organism	Dose	sc	po	sc	po
S. aureus Smith	Single[a]	0.46	0.25	1.25	17
S. aureus Smith	Divided[b]	1.0	0.38	1.8	28
E. coli 8	Single[a]	7.4	8.5	130	>1000
E. coli 8	Divided[b]	17	17	60	2000
P. mirabilis 13	Single[a]	64		14	
P. mirabilis 13	Divided[b]	84		19	

[a] 1 h postinfection. [b] 1 and 5 h postinfection.

should represent unchanged antibiotic.

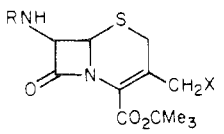
Taking into account both in vitro activity (Table I) and the blood level data (Table II), the *p*-carboxybenzylcephalosporin (**18**) appeared to be the most promising of the new cephalexin analogues. It was therefore tested against intraperitoneal infection in mice in comparison with cephalexin, and the results are given in Table III. By the subcutaneous route, compound **18** proved to be slightly less active than cephalexin against *S. aureus* Smith and significantly less active against *Escherichia coli*, but it was more active than cephalexin against *Proteus mirabilis*. As expected, **18** showed only poor activity by the oral route.

Experimental Section

Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian A-60 instrument for solutions in $CDCl_3$ with $SiMe_4$ as internal standard unless stated otherwise. Mass spectra were determined with a AEI MS9 machine. Compounds shown as having been analyzed either gave figures for the elements cited correct to within ±0.4 of the theoretical values or else gave accurate mass measurements correct to within ±5 ppm. Merck silica gel GF 254 was used for TLC and Merck silica gel H for column chromatography, with ethyl acetate–petroleum ether as eluent. Petroleum ether refers to the fraction of bp 60–80 °C.

Since most of the syntheses followed a lengthy but now standard[1-3] route (Scheme I), no attempt was made to characterize all the intermediates fully, but normal spectroscopic data were satisfactory. Several typical examples of the first two stages of Scheme I, namely, preparation of the secopenicillanates (**3**) and their oxidative conversion into 4-alkynylthioazetidin-2-ones (**4**), have been described.[4] In every sequence, at least one cephem ester (**9**, **10**, or **11**) was fully characterized as summarized in Table IV. All *tert*-butyl ceph-3-em-4-carboxylates showed λ_{max} (EtOH) 263–273 nm and ν_{max} ($CHCl_3$) 1770–1790 cm^{-1} (β-lactam CO) and 1700–1720 cm^{-1} (ester CO). In the final preparative step TFA treatment[3] removed both the *tert*-butyl ester and the *N*-*tert*-butoxycarbonyl-protecting groups to give cephalexin analogues **12** as their TFA salts, which showed one zone of antibacterial activity on biochromatograms (BuOH–EtOH–H_2O and/or BuOH–AcOH–H_2O) and gave the expected IR and UV spectra.

Table IV. Intermediate *tert*-Butyl Ceph-3-em-4-carboxylates



Compd	Precursor for	R[a]	X[a]	Mp, °C (solvent)[b]	Formula	Analyses
27	14	Ph_3C	C_6H_4-*o*-CF_3	Amorphous	$C_{38}H_{35}F_3N_2O_3S$	M^+
28	14	PhCHCO(NH(Boc))	C_6H_4-*o*-CF_3	110-111 (EA-P)	$C_{32}H_{36}F_3N_3O_6S$	M^+
29	15	Ph_3C	C_6H_4-*p*-SO_2NMe_2	185-186 (M)	$C_{39}H_{41}N_3O_5S_2$	C, H, N, S
30	15	PhCHCO(NH(Boc))	C_6H_4-*p*-SO_2NMe_2	156-157 (EA-P)	$C_{33}H_{42}N_4O_8S_2$	C, H, N, S
31	16	PhCHCO(NH(Boc))	C_6H_4-*o*-CO_2An	157-159 (Tr E)	$C_{40}H_{45}N_3O_9S$	C, H, N, S
32	17	Ph_3C	C_6H_4-*m*-CO_2An	171-172 (C-E)	$C_{46}H_{44}N_2O_6S$	C, H, N, S
33	18	Ph_3C	C_6H_4-*p*-CO_2An	101-102 (M)	$C_{46}H_{44}N_2O_6S$	C, H, N, S
34	18	PhCHCO(NH(Boc))	C_6H_4-*p*-CO_2An	152-154 (Tr E)	$C_{40}H_{45}N_3O_9S$	C, H, N, S
35	19	H	THP	Amorphous	$C_{17}H_{26}N_2O_4S$	M^+
36	19	PhCHCO(NH(Boc))	THP	Amorphous	$C_{30}H_{41}N_3O_7S$	M^+
37	20	Ph_3C	1-Me-2-pyrrolyl	167-170 (M)	$C_{36}H_{37}N_3O_3S$	M^+
38	20	H	1-Me-2-pyrrolyl	Amorphous	$C_{17}H_{23}N_3O_3S$	M^+
39	21	H	2-Pyridyl	Amorphous	$C_{17}H_{21}N_3O_3S$	M^+
40	21	PhCHCO(NH(Boc))	2-Pyridyl	Amorphous	$C_{30}H_{36}N_4O_6S$	M^+
41	23	H	4-Me-3-pyridyl	155-157 (C-E)	$C_{18}H_{23}N_3O_3S$	C, H, N, S
42	23	PhCHCO(NH(Boc))	4-Me-3-pyridyl	Amorphous	$C_{31}H_{38}N_4O_6S$	M^+
43	24	H	5(CO_2CMe_3)-3-pyridyl	156-158 (C-P)	$C_{22}H_{29}N_3O_5S$	M^+
44	25	Ph_3C	CO_2Me	109-110 (Tr E)	$C_{33}H_{34}N_2O_5S$	M^+
45	26	PhCHCO(NH(Boc))	CH_2OTHP	Amorphous	$C_{31}H_{43}N_3O_8S$	M^+
46	26	PhCHCO(NH(Boc))	CH_2OCOMe	Amorphous	$C_{28}H_{37}N_3O_8S$	M^+

[a] Abbreviations: An = *p*-methoxybenzyl, Boc = *tert*-butoxycarbonyl, THP = 2-tetrahydropyranyl. [b] Crystallized from C = chloroform, E = ethanol, EA = ethyl acetate, M = methanol, P = petroleum ether, Tr E = trituration with ether. All compounds gave satisfactory NMR spectra and were homogeneous by TLC.

The exact sequence (Scheme I) previously described[3] for preparation of **13** and **22** was used to prepare **14**, **15**, **19**, **21**, and **23**.

3-(Carboxybenzyl)-7-β-(D-phenylglycylamino)ceph-3-em-4-carboxylic Acids (16–18). The following route to **18** is typical.

3-*p*-(*p*-Methoxybenzyloxycarbonyl)phenylprop-2-ynol. This was prepared from copper(I) 3-(tetrahydropyran-2-yloxy)prop-1-ynide (33.2 g, 0.164 mol) and *p*-methoxybenzyl 4-iodobenzoate (60.5 g, 0.164 mol) by the general method of Harris et al.[4] The product (35.7 g, 74%) had mp 104.5–105.5 °C; ν_{max} 3590, 3400, 1705, 1609, 1520 cm^{-1}.

2-*p*-(*p*-Methoxybenzyloxycarbonyl)phenylprop-2-ynyl Bromide. To a suspension of the above alcohol (33 g, 0.111 mol) and PPh_3 (33 g, 0.126 mol) in dry benzene (150 mL) was added CBr_4 (45 g, 0.136 mol). The mixture was stirred and the exothermic reaction controlled by cooling so that the internal temperature did not exceed 40–45 °C. After 10 min the clear solution was evaporated and the residual oil chromatographed to give the bromide (26 g, 90%): needles (from AcOEt–petroleum ether); mp 60 °C; ν_{max} ($CHCl_3$) 1720, 1610, 1590, 1520 cm^{-1}. Anal. ($C_{18}H_{15}BrO_3$) C, H, Br.

1-(1-Benzyloxycarbonyl-2-methylprop-1-enyl)-4-[3-*p*-(*p*-methoxybenzyloxycarbonyl)phenylprop-2-ynylthio]-3-(triphenylmethylamino)azetidin-2-one. The above bromide (20 g) and benzyl 6β-(triphenylmethylamino)penicillanate (**1**, 28 g, 51 mmol) in dry THF (250 mL) were treated with freshly powdered NaOH (2.5 g, 62.5 mmol) and stirred at room temperature for 24 h. The mixture was then diluted with EtOAc, washed, dried, and evaporated. Chromatography of the residue gave the 1,2-secopenicillanate (26.7 g, 63%) as a foam: ν_{max} ($CHCl_3$) 1765, 1720 cm^{-1}. Anal. ($C_{52}H_{46}N_2O_6S$) C, H, N, S.

4-[3-*p*-(*p*-Methoxybenzyloxycarbonyl)phenylprop-2-ynylthio]-3-(triphenylmethylamino)azetidin-2-one. The above secopenicillanate (5 g, 6.05 mmol) in DMF (15.1 mL) and pyridine (15.1 mL) containing water (3.05 mL) was cooled to −20 °C and stirred while freshly powdered $KMnO_4$ (1.43 g, 9 mmol) was added portionwise at such a rate that the temperature did not exceed 0 °C. The mixture was stirred for 1 h at −20 °C, then diluted with EtOAc (40 mL) and water (40 mL), and treated with SO_2 until the MnO_2 had dissolved. The organic phase was separated, washed with 1 N HCl followed by $NaHCO_3$ solution, dried, and evaporated. Chromatography of the residue gave unchanged secopenicillanate (0.61 g) and the azetidinone (1.1 g, 29%) as a foam: ν_{max} ($CHCl_3$) 3400, 1762, 1710, 1610 cm^{-1}.

1-(1-Hydroxy-1-*tert*-butoxycarbonylmethyl)-4-[3-*p*-(*p*-methoxybenzyloxycarbonyl)phenylprop-2-ynylthio]-3-(triphenylmethylamino)azetidin-2-one. A solution of the above azetidinone (13.2 g, 0.021 mol) and *tert*-butyl glyoxylate (21 g, 0.16 mol) in benzene (250 mL) was boiled under reflux with provision for the removal of water. After 5 h the solution was cooled, washed, dried, and evaporated. Chromatography of the residue gave the α-hydroxy ester as a mixture of epimers (11.68 g, 74%): a foam; ν_{max} ($CHCl_3$) 3500, 1770, 1730, 1610 cm^{-1}.

1-(1-*tert*-Butoxycarbonyl-1-triphenylphosphoranylidenemethyl)-4-[3-*p*-(*p*-methoxybenzyloxycarbonyl)phenylprop-2-ynylthio]-3-(triphenylmethylamino)azetidin-2-one. The above α-hydroxy ester (5 g, 6.5 mmol) in dry THF (50 mL) containing 2,6-lutidine (2.38 g, 22 mmol) was cooled to −10 °C and treated dropwise with $SOCl_2$ (2.32 g, 19.5 mmol) during 15 min. After a further 3 min the precipitated solid was removed by filtration and the filtrate was evaporated to leave the crude α-chloro ester (4.0 g) as a foam: ν_{max} ($CHCl_3$) 1770, 1730 cm^{-1}. This was dissolved in dry dioxane (50 mL) containing 2,6-lutidine (3.4 g, 32 mmol) and triphenylphosphine (3.4 g, 13 mmol) and stirred under N_2 at 50 °C for 15 h and then filtered and the filtrate was evaporated. Chromatography of the residue gave the phosphorane (4.25 g, 64%) as a foam: ν_{max} ($CHCl_3$) 1755, 1715, 1635, 1610 cm^{-1}.

1-(1-*tert*-Butoxycarbonyl-1-triphenylphosphoranylidenemethyl)-4-[3-*p*-(*p*-methoxybenzyloxycarbonyl)phenyl-2-oxopropylthio]-3-(triphenylmethylamino)azetidin-2-one. The above acetylene (7.86 g) in piperidine (50 mL) was stirred at 50 °C for 4 h and then evaporated in vacuo. The residue was dissolved in EtOAc, washed successively with 0.1 N HCl, H_2O, and $NaHCO_3$ solution, dried, and evaporated. Chromatography gave the ketone (6.5 g, 82%) as a foam: ν_{max} ($CHCl_3$) 1750, 1710, 1630, 1610 cm^{-1}.

***tert*-Butyl 3-[*p*-(*p*-Methoxybenzyloxycarbonyl)benzyl]-7β-(triphenylmethylamino)ceph-3-em-4-carboxylate (33).** This was obtained (82%) by heating the above ketophosphorane in refluxing dioxane under N_2 for 24 h.

***tert*-Butyl 7β-(*N*-*tert*-Butoxycarbonyl-D-α-phenylglycyl)amino-3-[*p*-(*p*-methoxybenzyloxycarbonyl)benzyl]ceph-3-em-4-carboxylate (34).** The cephem **33** (3.8 g, 5 mmol) in CH_2Cl_2 (10 mL) was detritylated by treatment at −20 °C with toluene-*p*-sulfonic acid (1 g, 6 mmol) in MeOH (5 mL). The solution was left at 0 °C for 16 h and then washed with $NaHCO_3$ solution. Evaporation of the organic layer gave the primary amine (1.75 g, 68%): mp 134–135 °C; ν_{max} ($CHCl_3$) 1775, 1710 cm^{-1}. This amine (250 mg, 0.5 mmol) in THF (2 mL) was added over 5 min to a cooled (−20 °C) THF solution of mixed anhydride prepared[3] from *N*-*tert*-butoxycarbonyl-D-α-phenylglycine (135 mg, 0.54 mmol) and $ClCO_2Me$ (50 mg, 0.53 mmol). The mixture was stirred for 2 h at −20 °C and then evaporated in vacuo. The residue was taken up in EtOAc and washed successively with 0.25 N HCl (2 × 10 mL), water, and $NaHCO_3$ solution. Evaporation of the dried solution followed by chromatography gave the protected cephalosporin **34** (193 mg, 53%): ν_{max} ($CHCl_3$) 3400, 1785, 1710 cm^{-1}.

3-(*p*-Carboxybenzyl)-7β-(D-α-phenylglycyl)aminoceph-3-em-4-carboxylic Acid (18). The triply protected compound **34** (86 mg) was dissolved in TFA (0.5 mL) and kept at room temperature for 10 min. The solution was then evaporated in vacuo and the evaporation thrice repeated after successive additions of dry toluene (5-mL portions) to leave the TFA salt of **18** as an amorphous solid (63 mg): ν_{max} (Nujol) 2300–2700, 1770, 1690 cm^{-1}.

3-(5-Carboxy-3-pyridylmethyl)-7β-(D-α-phenylglycylamino)ceph-3-em-4-carboxylic Acid (24). This was prepared by a similar reaction sequence to **18**, except that the final TFA treatment of **11** (X = 5-*tert*-butoxycarbonyl-3-pyridyl) required 3 h for complete deprotection [checked by running biochromatograms in BuOH–AcOH–H_2O (12:3:5) until only one zone of antibacterial activity (R_f 0.37) remained].

***tert*-Butyl 3-Methoxycarbonylmethyl-7β-(triphenylmethylamino)ceph-3-em-4-carboxylate (44).** (3*R*,4*R*)-4-Prop-2-ynylthio-3-(triphenylmethylamino)azetidin-2-one[4] (**4**, X = H; 9.3 g) was converted by the method of Scheme I into the phosphorane (**7**, X = H; 11.4 g, 65%) and the latter in THF was treated at −70 °C with *n*-butyllithium (2 equiv). After 5 min the mixture was saturated with CO_2, diluted with EtOAc, washed with dilute HCl, dried, and evaporated to small volume. Treatment with diazomethane in ether gave, after chromatography, the methyl ester **7** (X = CO_2Me) as a foam (30%): ν_{max} ($CHCl_3$) 2280, 1760, 1720, 1635 cm^{-1}. Treatment with piperidine at room temperature for 6 h, followed by usual work-up with aqueous acid, gave the ketone **8** (X = CO_2Me) as a foam (67%), ν_{max} ($CHCl_3$) 1755, 1720, 1635 cm^{-1}, which when refluxed in dioxane for 24 h gave the cephem **44** (Table IV) in 70% yield.

1-(1-Benzyloxycarbonyl-2-methylprop-1-enyl)-4-(3-iodoprop-2-ynylthio)-3-(triphenylmethylamino)azetidin-2-one (3, X = I). A solution of $AgNO_3$ (12.6 g, 74 mmol) in $CHCl_3$ (100 mL) and pyridine (50 mL) was stirred and cooled in an ice bath, while iodine monochloride (4.2 mL, 83 mmol) in $CHCl_3$ (50 mL) was added over 5 min. AgCl was removed by centrifugation and washed with pyridine (50 mL). The combined solutions, containing iodonium nitrate, were treated with the 1,2-secopenicillanate[4] (**3**, X = H, 28 g, 47.8 mmol) in $CHCl_3$ (50 mL) and stirred at room temperature for 4 h. The mixture was evaporated in vacuo and the residue taken up in EtOAc, washed, dried, and again evaporated. Chromatographic purification gave the iodoacetylene **3** (X = I, 25.9 g, 76%) as a yellow foam: ν_{max} ($CHCl_3$) 2980, 1765, 1715, and 1630 cm^{-1} (found M^+ 712.1291; $C_{37}H_{33}IN_2O_3S$ requires M 712.1258).

1-(1-*tert*-Butoxycarbonyl-1-triphenylphosphoranylidenemethyl)-4-(3-iodoprop-2-ynylthio)-3-(triphenylmethylamino)azetidin-2-one (7, X = I). This was prepared (10% overall) from **3** (X = I) according to Scheme I.

***tert*-Butyl 3-(1-Methyl-2-pyrrolylmethyl)-7β-(triphenylmethylamino)ceph-3-em-4-carboxylate (37).** *N*-Methylpyrrole (0.5 g, 6.2 mmol) and tetramethylethylenediamine

(0.87 g, 5.7 mmol) in dry ether were treated with *n*-butyllithium (1 equiv) at reflux for 1 h. The solution of 2-lithio-*N*-methylpyrrole was stirred with copper(I) bromide (1.2 g) for 90 min at room temperature to give a brown suspension of 2-cuprio-*N*-methylpyrrole. This was treated with the iodoacetylene 7 (X = I, 5.0 g, 6.5 mmol) in THF (20 mL) and stirred at room temperature overnight. The mixture was diluted with benzene, the solids were removed by centrifugation, and the supernatant solution was concentrated and then chromatographed to give the pyrrolylacetylene 7 (X = 1-methyl-2-pyrrolyl) (28%): ν_{max} ($CHCl_3$) 2980, 1750, and 1630 cm^{-1}. Refluxing in piperidine for 12 h, followed by the usual aqueous work-up, gave the ketone 8 (X = 1-methyl-2-pyrrolyl, 59%): ν_{max} ($CHCl_3$) 2980, 1760, and 1635 cm^{-1}. Cyclization by refluxing in dioxane for 30 h gave the cephem 37 (Table IV) in 83% yield.

***tert*-Butyl 7β-Amino-3-(1-methyl-2-pyrrolylmethyl)-ceph-3-em-4-carboxylate (38).** For this acid-sensitive compound the following exceptionally mild detritylation procedure was preferred. The trityl derivative 37 in redistilled MeOH was stirred with an equal weight of pyridine hydrochloride for 4 days; then the solvent was evaporated and the residue dissolved in EtOAc. The solution was washed with aqueous $NaHCO_3$, dried, and evaporated, and the residue was chromatographed to give the primary amine 38 (81%).

***tert*-Butyl 3-(2-Acetoxyethyl)-7β-(*N*-*tert*-butoxycarbonyl-D-α-phenylglycyl)aminoceph-3-em-4-carboxylate (46).** The *O*-tetrahydropyranyl derivative 45 (106 mg, 0.17 mmol, synthesized according to Scheme I) was treated with toluene-*p*-sulfonic acid (35 mg, 0.2 mmol) in isopropenyl acetate (4 mL) at 0 °C for 4 h and then at room temperature for a further 4 h. EtOAc was added and the solution washed with dilute aqueous $NaHCO_3$, followed by brine. Evaporation of the dried solution, followed by chromatography, gave the cephem 46 (Table IV) in 25% yield.

Supplementary Material Available: High-resolution mass spectral data, NMR data, and elemental composition data (4 pages). Ordering information is given on any current masthead page.

References and Notes

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Notes

Structure and Biological Activity of (−)-[^{3}H]Dihydroalprenolol, a Radioligand for Studies of β-Adrenergic Receptors

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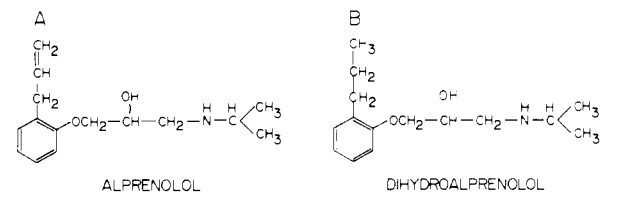
(−)-Alprenolol is a potent competitive β-adrenergic antagonist. "(−)-[^{3}H]Alprenolol", a radioactive form of this agent produced by catalytic reduction with tritium, has recently been used successfully as a radioligand for direct studies of β-adrenergic receptors. In this communication it is documented that the compound formed by catalytic reduction of (−)-alprenolol with tritium gas is the saturated product (−)-[^{3}H]dihydroalprenolol in which tritium is added across the double bond and exchanged into the adjacent benzylic position. No exchange into the aromatic ring was observed. These conclusions were substantiated by results obtained on hydrogenation and deuteration of (−)-alprenolol. The biological activity of (−)-[^{3}H]dihydroalprenolol, dihydroalprenolol, and alprenolol was also shown to be identical as assessed by direct ligand binding and inhibition of catecholamine-stimulated adenylate cyclase.

Recently reports from several laboratories have indicated the feasibility of directly studying β-adrenergic receptors by radioligand binding techniques. Several agents have been used. These have included [^{3}H]-(±)-propranolol,[2,3] "(−)-[^{3}H]alprenolol",[4-13] and (±)-[^{125}I]hydroxybenzylpindolol.[14-16] (−)-Alprenolol [1-(2-allylphenoxy)-3-isopropylamino-2-propanol] contains an olefinic bond in the hydrocarbon side chain on the benzene ring. Since the radioligand is produced by catalytic reduction of the molecule with tritium, it has seemed likely that "(−)-[^{3}H]alprenolol" was in all probability (−)-[^{3}H]dihydroalprenolol [[*propyl*-2,3-^{3}H(N)]-1-(2-propylphenoxy)-3-isopropylamino-2-propanol]. This has not previously been documented.

In this communication we present data which (1) verify that the structure of tritiated "(−)-alprenolol" formed by catalytic reduction with tritium is in fact (−)-[^{3}H]dihydroalprenolol and (2) document that the biological activity of (−)-alprenolol and (−)-dihydroalprenolol is identical.

Chart I



Discussion

(−)-Alprenolol (see Chart I) has been catalytically reduced with hydrogen, deuterium, and tritium to yield the corresponding saturated derivatives. The reduced materials have indistinguishable UV spectra and are homogeneous and indistinguishable by TLC. The R_f values