SYNTHESIS OF REGIOSPECIFICALLY SUBSTITUTED PYRIMIDYL DERIVATIVES AND THEIR INCORPORATION INTO PENEMS

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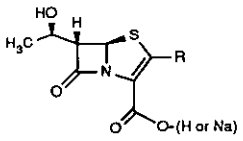
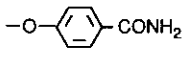
Abstract - Syntheses of regiospecifically substituted pyrimidines are described. Depending on the reaction conditions, N_1- or N_3-substituted pyrimidines are obtained. It has been shown that substitution on uracil under Mitsunobu conditions yields N_1-substituted products. Incorporation of these derivatives into the penem nucleus gives penem antibiotics with extremely long half-lives.

Penems 1 are highly potent, broad-spectrum β-lactam antibiotics, closely related to the penicillins, the cephalosporins and the carbapenems. Since the publication of the first penem synthesis by the Woodward-CIBA group in the 1970's[1], chemists have been endeavouring to discover an economical route for the total synthesis of penems possessing a high degree of antibiotic activity[2]. These efforts have led to a series of development compounds, which are or have been in the past under investigation by various companies (Table 1).

Table 1



1

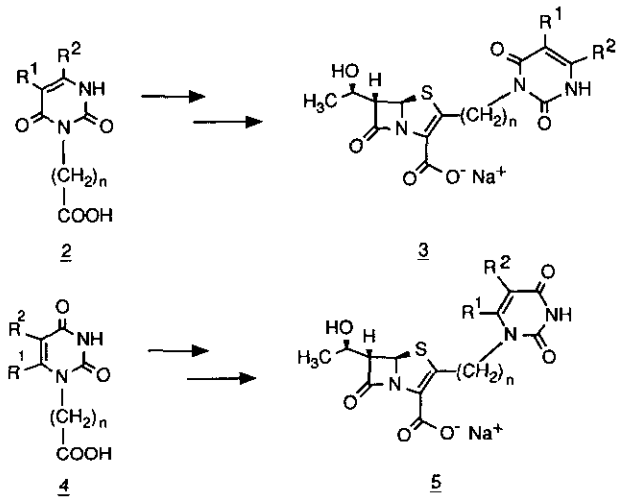
R	Code/Company	
$-SCH_2CH_3$	SCH 29482	Schering
$-CH_2OCONH_2$	FCE 22101	Carlo Erba
$-SCH_2CH_2OCONH_2$	SCH 34343	Schering
$-CH_2NH_2$	CGP 31608	Ciba-Geigy
	SUN 5555	Suntory
	HRE 664	Hoechst

The outstanding compound in this list is CGP 31 608; of all the penems prepared and known from published data, CGP 31 608 is the only one displaying not only excellent activity against anaerobes, Gram-positive and the "normal" Gram-negative bacteria, but also against Pseudomonas aeruginosa. For this reason, CGP 31 608 can justly be described as a genuine broad-spectrum penem antibiotic, only comparable to the carbapenems[3].

Besides good antibacterial activity, an important criterion of the clinical efficacy of β-lactam antibiotics is the duration of their sojourn in the blood plasma. The half-lives of these substances in human plasma is usually limited, ranging from 1 to 2 hours. One of our principal objectives has consequently been to synthesize a new

penem with a longer half-life than the existing representatives of this class, the other semisynthetic penicillins and the cephalosporins.

Scheme 1

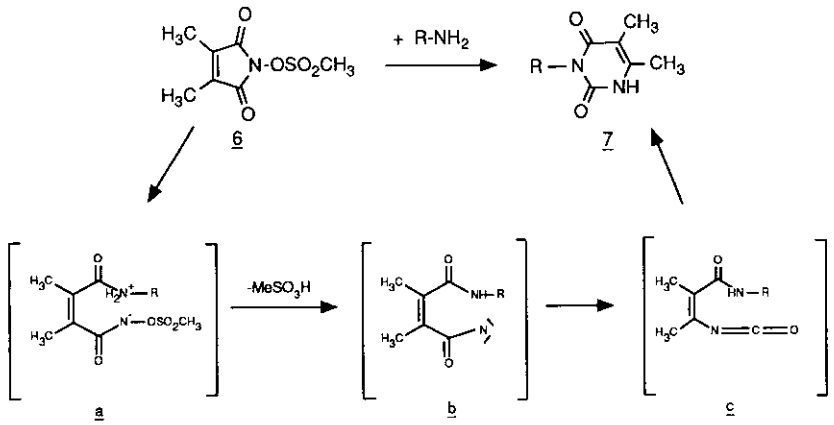


As rational thinking does not hold the key to success in the design of penem antibiotics with long plasma half-lives, we were pleased, during our systematic derivatization work on CGP 31 608, to discover the pyrimidyl penems 3 and 5, (from 2 and 4 respectively) both possessing the desired properties[4,5].

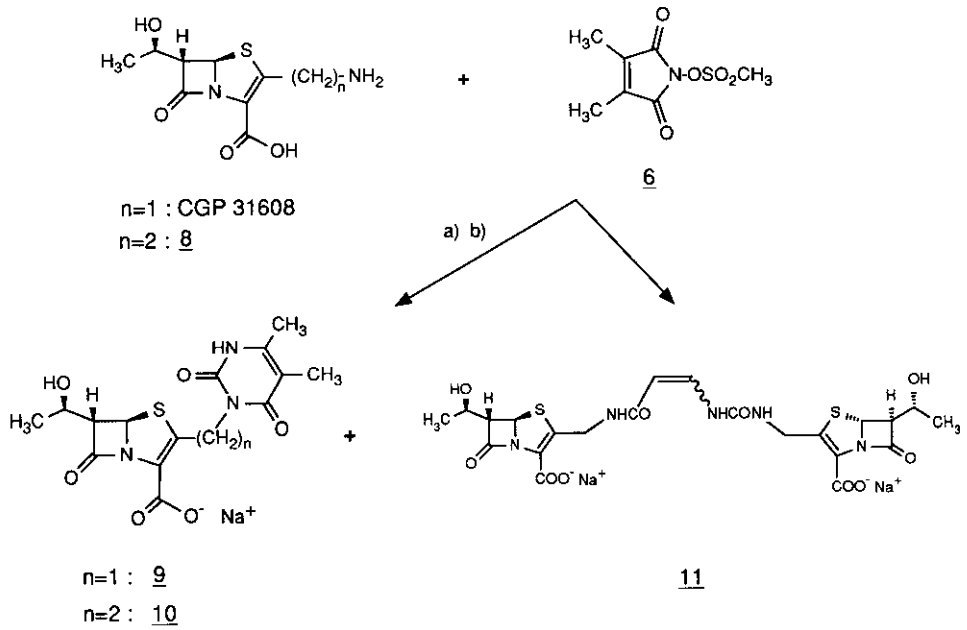
CGP 31 608, bearing an alkylamino group, proved to be an interesting lead compound that reacts easily with a wide variety of amino reagents, giving acyl derivatives, carbamates, urethanes, etc. The maleic acid anhydride derivative 6 [6] reacts, in a rather peculiar fashion, with primary amino groups to yield 7 (Scheme 2). The reaction (Scheme 2) is thought to proceed by way of the Lossen rearrangement[7] of the cis-oriented hydroxamic acid intermediate a to the isocyanate c. The subsequent cyclization affords an elegant synthesis of regiospecifically substituted pyrimidyl derivatives. By analogy with the reaction 6 → 7, CGP 31 608 and its homologue 8 reacted with 6 to give the penem derivatives 9 and 10 (Scheme 3). For n = 1, 11 was a by-product. Albeit isolated as an isomeric mixture only in a modest yield, its existence supports the proposed reaction mechanism of Scheme 2.

The pyrimidyl penem 9, the structure of which was further confirmed by an independent synthesis (see below), could be prepared in gram quantities in an average, non-optimalized yield of 50-60 %, starting from CGP 31 608. The nmr spectrum of 9 in D_2O (Table 3), FAB/MS (M-H=366) and ^{13}C-nmr were in agreement with the proposed structure. Biological data on 9, e.g. elimination half-life in mouse plasma (41 min), protein binding (90 % in human serum) and plasma concentration (19.8 µg/ml 50 min after administration) justified the further exploration of the regiospecifically substituted pyrimidyl penems in order to elucidate the relations between the biological properties and the substitution pattern of the pyrimidyl ring.

Scheme 2



Scheme 3



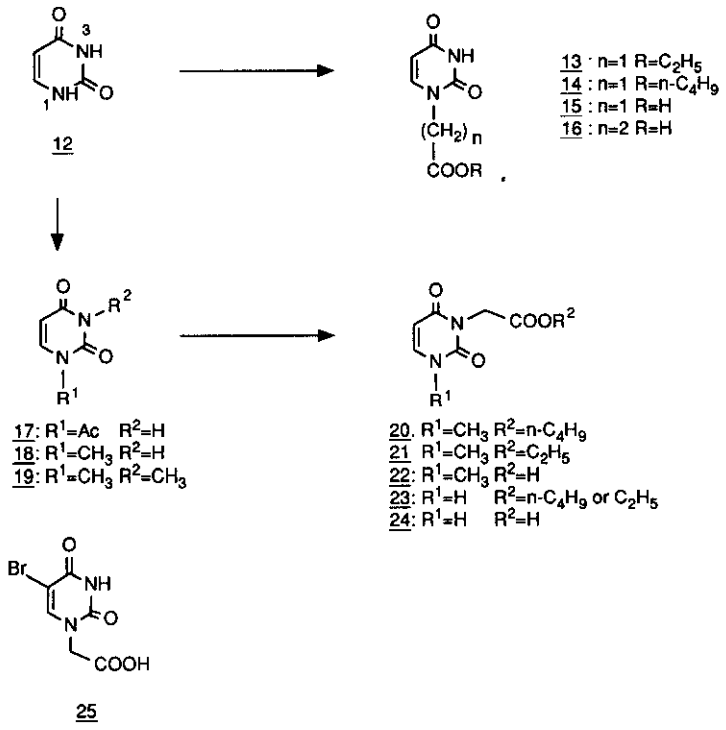
a) H_2O/pH=8-8 5/4-5 h (tlc control)

b) chromatography on reversed- phase silica gel in H_2O, ca 50%

Scheme 4 summarizes the reaction sequence carried out with uracil 12. Our primary target compounds were the "amide"-acid 15 and the "imide"-acid 24. Alkylations of uracil 12 are known to yield predominantly N_1-substituted products. Indeed, the synthesis of 15 was accomplished by the published procedure[8].

Alternatively, alkylation of 12 with ethyl iodoacetate gave the ester 13, which was converted to 15 by hydrolysis with conc. HCl.

Scheme 4



Reaction conditions

14,20,23	$HOCH_2COO$-n-C_4H_9/$P(C_6H_5)_3$/DEAD/THF/control by tlc (23 . R^2 = n-C_4H_9)
13,21,23	: $ICH_2COOC_2H_5/K_2CO_3$/DMSO/RT or heat (23 $R^2 = C_2H_5$)
15	$ClCH_2COOH$/KOH/15 min/100° C,[8] or conc HCl/2 5 h/100° C (from 13)
16	CH_2=CH-CN/1n NaOH/15h[21]
17	: Ac_2O-Py 1 1/4 h/90° C
18,19	CH_3I/K_2CO_3/DMSO/0° C
22,24	4n HCl/100° C/tlc control or NaOH/H_2O
25	Br_2/H_2O/1 h/ RT[9]

The synthesis of penem 5 (n=1; R^1=R^2=H), starting from silver salt 26[9] and acid chloride 27 via phosphorane 28 and penem ester 29, is described in Scheme 5[10] . To our surprise, the nmr spectroscopic data of 5 were fully identical with the published data of the penem resulting from deprotection of the ester 31, postulated (Scheme 6) as the product of a Mitsunobu reaction between uracil 12 and 30[11,12].

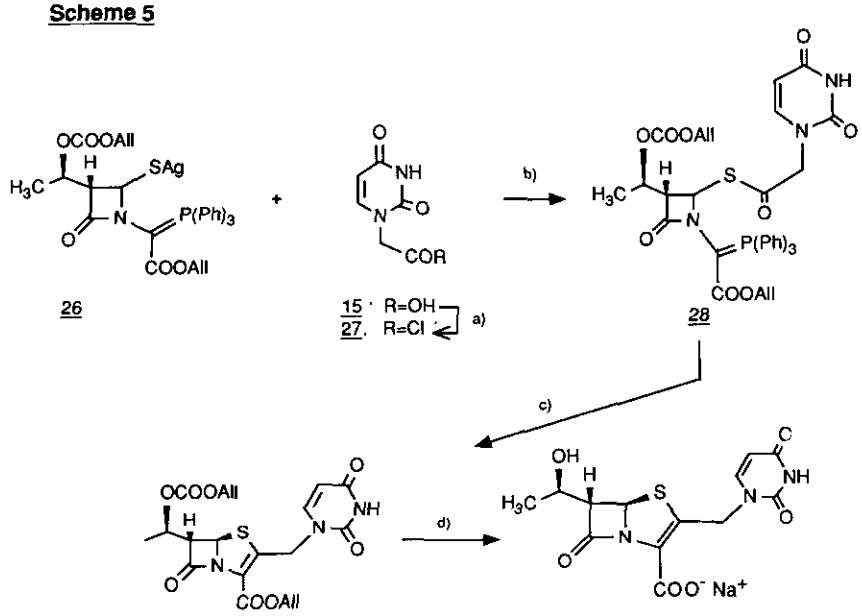
We therefore began to investigate the reactivity of uracil 12, a multifunctional substrate, under Mitsunobu conditions (glycolic acid n-butyl ester, triphenylphosphine, DEAD). Careful chromatography of the resultant complex reaction mixture led to the isolation of only one monosubstituted uracil ester 14 (26 %; mp: 111-112°C), which after hydrolysis gave the acid 15, identical in every respect with the product of the reaction

sequence 12→15, or 12→13→15. The N_3-substituted product 23 (R_2=n-C_4H_9) was not detectable.

O,O-Bisalkylated by-products resulting from the reaction with the respective enolates and N,N-bisalkylated products were identified by nmr spectroscopy, but could not be separated[14].

In the literature, the synthesis of the isomeric uracil acid 24 starting from cytosine is reported[15], but we were not able to reproduce the described procedure. We therefore prepared the N_1-acetyluracil 17 [16], carrying an easily removable blocking group. Mitsunobu alkylation of 17 by analogy with the preparation of 14 gave an inseparable mixture of deacylated esters 23 (R^2=n-C_4H_9; major) and the isomeric compound 14 (minor; ratio by nmr approx. 4:1). However, 17 on reaction with ethyl iodoacetate (K_2CO_3/DMSO/RT/6h) gave a 32% yield of pure 23 (R^2=C_2H_5; mp: 141°C), easily separated from the N,N-bisalkylated derivative (16%; not shown in the

Scheme 5

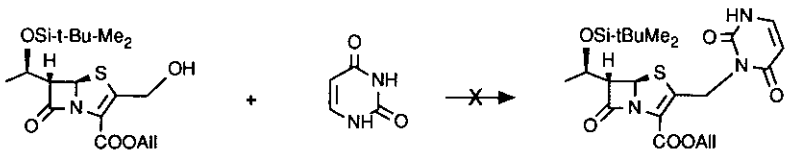


Reaction conditions
a) for preparation of acid chlorides see[13]
b) CH_2Cl_2/pyridine/RT
c) Toluene/ heat
d) Pd°/1,3-dimethylbarbituric acid/THF/RT
All = -$CH_2CH=CH_2$

scheme). The acid 24 was obtained as usual by hydrolysis from 23 (R^2=C_2H_5).The nmr spectroscopic data of the esters 14 and 23 (R^2=C_2H_5) and of the corresponding acids 15 and 24 are presented in Table 2.

By analogy with the synthesis of 5 (Scheme 5), 24 served as starting material for the synthesis of the

Scheme 6



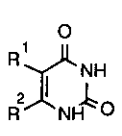
30 12 31

Penem

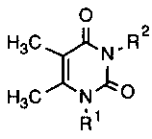
regioisomeric penem, which results from the deprotection of 31 (Scheme 6; nmr data see table 3). Thus it was demonstrated that 31 is clearly not the main product of a Mitsunobu reaction of 30 and 12 [17,18].

Further transformations of uracil 12 are shown in Scheme 4. Methylation with CH_3I/K_2CO_3 in DMSO led to a mixture of 18 (major; mp 231-234°C) and 19 (minor; mp 118-121°C)[19,20], separated by chromatography. Preparation of 20-22, 16[21] and 25[8] followed standard procedure.

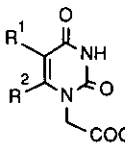
Scheme 7



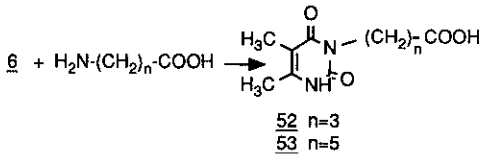
	R^1	R^2
38	CH_3	CH_3
39	CH_3	H
40	F	H
41	H	CH_3



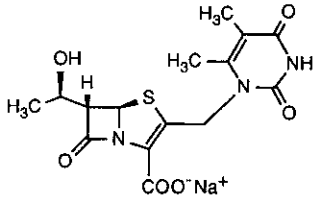
	R^1	R^2
42	$CH_2COOC_2H_5$	H
43	CH_2COOH	H
44	H	$CH_2COOC_2H_5$
45	H	CH_2COOH
46	$CH_2COOC_2H_5$	$CH_2COOC_2H_5$



	R^1	R^2	R^3
47	F	H	C_2H_5
48	F	H	H
49	CH_3	H	H
50	H	CH_3	n-C_4H_9
51	H	CH_3	H



52 n=3
53 n=5



54

In Scheme 7, additional derivatives are given. Starting from commercially available 5,6-dimethyluracil 38, the esters 42 and 44 were prepared ($ICH_2COOC_2H_5/K_2CO_3$/DMSO/50°C/15 h) and separated by chromatography. In this reaction, N,N-dialkylated uracil 46 (ir: 1749, 1702, 1655 cm^{-1} in CH_2Cl_2; mp 92 - 93°C) was the main product. Acids 43 and 45, obtained from 42 and 44 by acidic hydrolysis, gave the penems 54 and again 9.

Thus, the synthesis of 9, accomplished in an independent way, enabled us to assign the correct regiochemistry of the substitution in 42 and 44 and the corresponding acids 43 and 45, whose spectroscopic data alone do not permit unambiguous structure assignment (see Tables 2 and 3)[22].

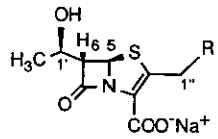
Compound 49 was prepared according to the published procedure[8] starting from thymine 39; interestingly enough, we were not able to prepare the isomer 51 (from 41) by the same method. Again, Mitsunobu's procedure yielded 50 and thereafter the desired acid 51. Alkylation on 5-fluorouracil (40) is reported to yield mixtures of monosubstituted and disubstituted products on nitrogen, depending on the reaction conditions[24]. By our procedure ($ICH_2COOC_2H_5/K_2CO_3$/DMSO/50°), an easily separable, approx. 2:1 mixture of 47 and N,N-dialkylated fluorouracil (not shown in the scheme) was obtained. 48 was prepared from 47 by hydrolysis. Finally, following the reaction sequence depicted in Scheme 2, the acids 52 (44 %; mp 182 - 184°C) and 53 (57 %; mp 185 - 187°C) were prepared.

Table 4 summarizes some of the kinetic parameters of the uracil penems 3 and 5. The synthesis proceeds in the way outlined in Schemes 3 and 5. The results indicate that the long half-life is not primarily dependent on the N_1- or N_3-substitution pattern of the pyrimidine ring, but on the spacer -$(CH_2)_n$- and on the substituents on the ring itself.

Table 2 ^{1}H-Nmr spectroscopic data of 14,23,42,44 ($CDCl_3$) and 15, 24, 43, 45 (DMSO-d_6; 360 MHz)

	COOH	NH	H_3C-C(6) H-C(6)	H_3C-C(5) H-C(5)	2H-C(1')
14	-	8.79	7 12	5.77	4.47
23	-	9.05	7 09	5.73	4.43
42	-	8 41	2 18	1 98	4.65
44	-	9 86	2.16	1 93	4.68
15	13.15	11.35	7 61	5.60	4.41
24	12.95	11 28	7.51	5.67	4.41
43	13.18	11 35	2.13	1 83	4 55
45	12 90	11.06	2 08	1.78	4.41

Table 3 ^{1}H-Nmr spectroscopic data of penems 5, 31 (deprotected), 9 and 54 (D_2O; 360 MHz)



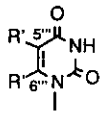
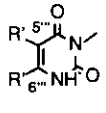
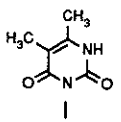
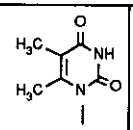
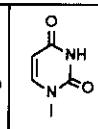
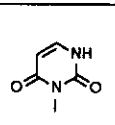
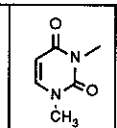
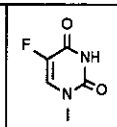
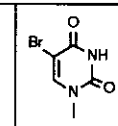
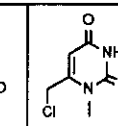
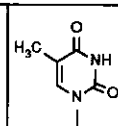
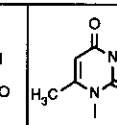
R	R'	H_3C-C(6'') H-C(6'')	H_3C-C(5'') H-C(5'')	H-C(5)	2H-C(1'')	H-C(1')	H-C(6)	H_3C-C(1')
	H (5)	7.70	5.86	5.65	5.30 / 4.98	4.24	3.92	1.30
	CH_3 (54)	2.33	1 93	5.63	5 48 / 5.22	4.24	3.91	1.29
	H (31)	7 54	5.89	5.61	5.43 / 5 02	4.23	3.90	1.28
	CH_3 (9)	2.21	1.89	5 59	5 43 / 5.03	4 23	3.88	1.28

Table 4 Pharmacokinetic parameters of selected pyrimidyl penems 3 and 5

Pyrimidyl side- chain														
n	1	2	3	5	1	1	2	1	1	1	1	1	1	1
$t_{1/2}$	41	5	5	5	25	7	4	7	16	5	20	13	9	6
PB	90	24	53	90	50	4	37	27	25	44	84	37	33	29
C(50)	19.8	-	<0.2	<0.2	29	-	<0.8	1.0	13.1	<0.4	21.8	<0.8	3.2	0.6

$t_{1/2}$: half-life in plasma of mice in min, PB protein binding in human serum in %, C(50) concentration of antibiotic in µg/ml in plasma of mice after 50 min.

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4. Full biological data on all penems mentioned in this paper have been presented at the 28th ICAAC[5] .
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6. Compound 6 was first prepared in our company by Dr. B. Müller of the Additives Division, who, together with Dr. M. Baumann of our Central Research Laboratories, explored the synthetic usefulness of this reagent (to be published). The authors thank Dr. M. Baumann for supplying our laboratory with 6.
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10. With the exception of penems 9 - 11, all penems mentioned in this paper were synthesized following the reaction pathway depicted in Scheme 5. This reaction sequence corresponds to the original Woodward procedure[1].
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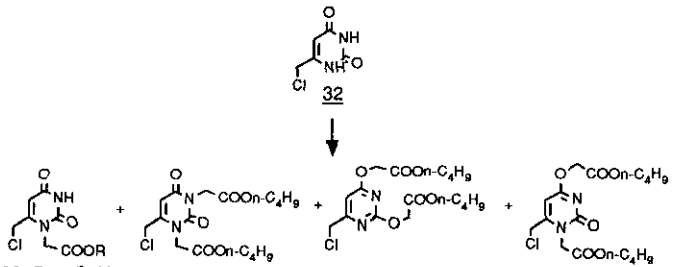
14. In all alkylation reactions of pyrimidines, following Mitsunobu's procedure or by standard methods, mixtures of products were obtained: e.g. the Mitsunobu reaction of 4-chlormethyluracil (32), a compound unsuitable for normal alkylation reactions because of its labile chlorine atom, gave the four products 33 - 36 depicted below. In this case, it was possible to separate and characterize the products by spectroscopic methods (^{1}H-,^{13}C-nmr, NOE; structure proposed for 36. 37 by hydrolysis from 33) whereas with the other pyrimidines only the N_1-monoalkylated main product was isolated.



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17. Although our conditions in the Mitsunobu reaction with uracils were not identical with the procedure described[11] , the exact reproduction of the published procedure with penem ester 30 and uracil 12 likewise gave penem 5 after deprotection.

18. It must be noted that structure assignment of the penems 5 and 31 (deprotected) based on ^{1}H-nmr data alone is difficult owing to the small difference in the chemical shifts of the two isomers (Table 3).

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