C(3)-ALKOXYCEPHEMS

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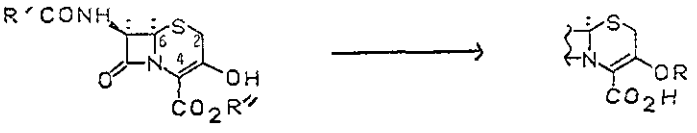
Abstract - The C(3)-hydroxycephems 1 undergo the Mitsunobu reaction to give a series of heretofore unavailable C(3)-alkoxycephems 2.

Several research groups have reported the synthesis and activity of C(3)-alkoxy cephems 2, R being Me, Et, n-Bu, and CH_2Ph.[1,2]



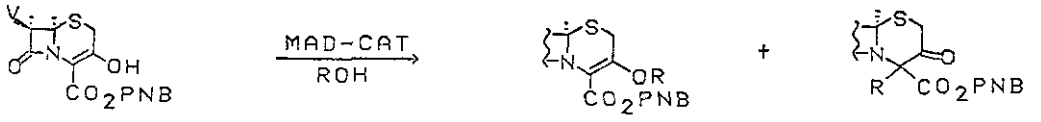
1 2

In general, these syntheses involve O-alkylation of the C(3)-hydroxycephem 1 with dimethyl sulfate, Meerwein's reagent, triazenes and the use of diazo derivatives.

We were interested in determining the in vitro and in vivo effect of lipophilic groups at the C(3)-position in the cephem molecule and needed a general, simple synthesis of C(3)-alkyl ethers.[3] Use of a Williamson type ether synthesis was found not to be applicable, leading instead to alkylation at C(4) using either the cephem sulfide 1 or its sulfoxide.

The Mitsunobu reaction consists of an oxidative-reductive-dehydrative coupling in the presence of trivalent phosphorous and an azodicarboxylate, generally involving an inter or intramolecular dehydration between an alcohol and an acidic component.[4] β-Keto esters have been reported to give low yields of the O-alkylated product. Mitsunobu, for example, reported the O-alkylation of ethyl acetoacetate under DEAD-CAT (diethyl azodicarboxylate-triphenylphosphine) conditions using n-propanol to give 18-37% O-alkylated product, depending on the reaction conditions.[4,5]

Application of the Mitsunobu reaction to the C(3)-hydroxycephem 3 using DEAD-CAT/MeOH gave, after silica chromatography, 60% C(3)-OMe, 5 (R=Me), identical to the product of 3 with diazomethane, and 10% C-alkylated product 4 (R=Me). The actual yield of the O-methylated product was somewhat higher, but we encountered problems separating the ether from the ethyl hydrazodicarboxylate by-product 8 (R'=Et). This problem was resolved by using dimethyl azodicarboxylate (MAD)[6], whose by-product is moderately soluble in water[7] and thus can be eliminated from concentrated ethyl acetate or methylene chloride by repeated (3-4 times) aqueous washings. In addition MAD appeared to be more reactive[7] than DEAD and gave higher yields in certain cases. We thus reacted the enol 3 under MAD-CAT conditions (THF or CH_2Cl_2, r.t., 20 min.) with a series of alcohols (methanol to n-octanol) to give 60-90% of the O-alkylated product 5 plus 10-15% of the C-alkylated product 4.

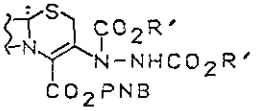


3 → 5 60-90% + 4 10-15%

V = $PhOCH_2CONH-$

PNB = $p-O_2NC_6H_4CH_2-$

MAD-CAT = $[MeO_2C-N=]_2/Ph_3P$



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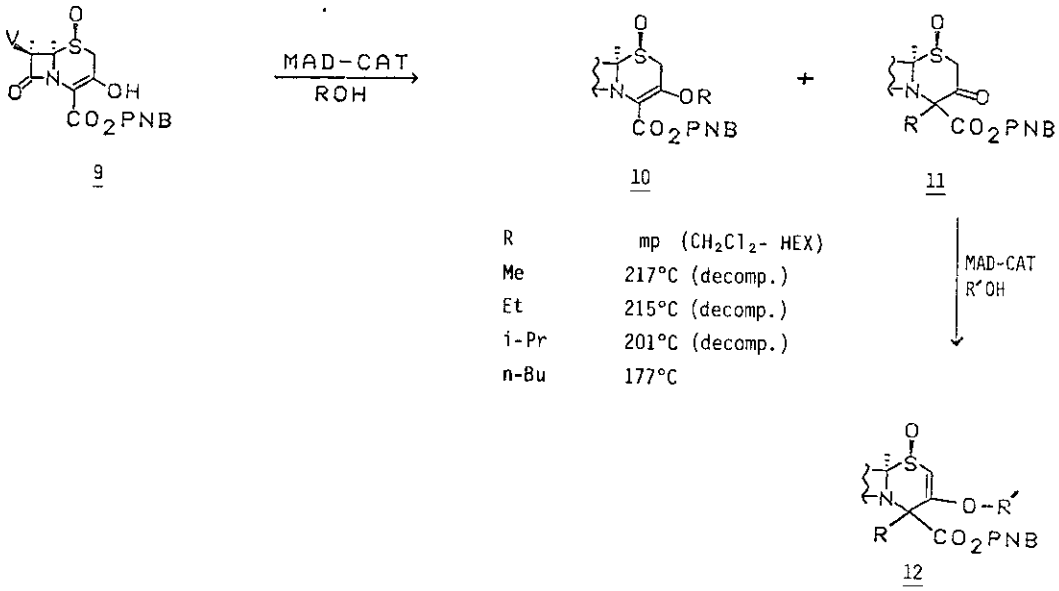
Ph_3PO + $[R'O_2CNH]_2$

7 8

The C-alkylated product 4 was distinguishable from 5 by NMR (the chemical shift of the methylene alpha to the oxygen vs. carbon, and the difference in the methylene at C(2), i.e. the ketone 4 showed a broad AB, ca. 3.0 and 3.7 ppm, J = 14 HZ, while 5 showed a singlet at ca. 3.4 ppm) and by ketone carbonyl absorption at ca. 1729 cm^{-1} in the i.r. spectrum.

The enol sulfoxide 9 under the MAD-CAT conditions also gave a mixture with a predominance of O-alkylation. This was determined by PBr_3 sulfoxide reduction followed by silica chromatography of the sulfides 4 and 5. The C-alkylated sulfoxide 11 further undergoes the Mitsunobu reaction to give the dialkylated Δ^2-derivative 12.



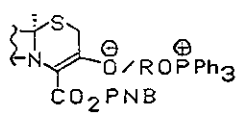
9 10 11

R	mp (CH_2Cl_2- HEX)
Me	217°C (decomp.)
Et	215°C (decomp.)
i-Pr	201°C (decomp.)
n-Bu	177°C

12

The C(3)-hydrazino cephem derivative 6 (R'=Me) was observed when secondary alcohols were used or alternatively, when no alcohol was present.

^{18}O-Methanol studies[8] show that the alkoxy phosphonium salt 13 derived from the alcohol is the intermediate for both O- and C-alkylation while the cephem alkoxy phosphonium salt 14 is believed to be the intermediate to the C(3)-hydrazinocephem 6.[5]



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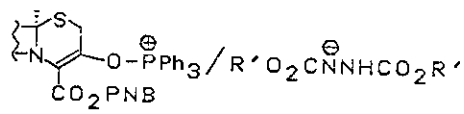
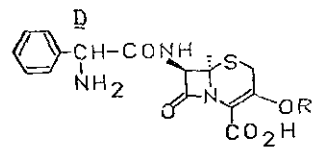


14

Use of trialkylphosphite[4]-MAD works equally well with the enol cephem. Thus the reaction of 3 with tri-n-butylphosphite-MAD gave 82% of O-alkylated n-Bu cephem 5, (R=n-Bu) while triethyl phosphite gave 77% of the O-ethyl derivative 5, (R=Et). PCl_5 cleavage[8] of the side chain of 5 followed by acylation and deblocking led to the phenyl glycine acids 15. Alternatively, use of the benzhydryl-t-boc-phenyl glycine-C(3)-enol as the Mitsunobu substrate followed by deblocking (HCO_2H) gave the same acids.

MIC data (Table I) on these acids in general show that as the lipophilic character at C(3) increases the gram positive acitivity is enhanced with concomitant loss in gram negative activity. A similar trend was seen with the C(3)-alkylcephems.[3]

TABLE I



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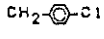
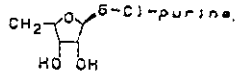
MIC (μg/ml)

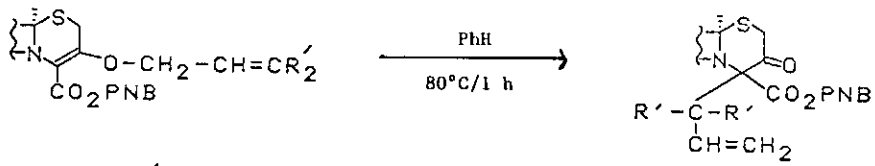
R	S. aureus.		S. epidermidis (amp^S)	H. influenzae	E. coli
	V41	S13E	222	CL	TEM
Me	128	64	8	8	8
Et	128	128	4	8	8
n-C_3	64	128	1	8	16
i-C_3	64	64	2	16	64
n-C_4	16	16	1	8	64
n-C_5	8	8	0.5	8	64
n-C_6	16	32	0.5	8	64
n-C_8	16	16	1	32	128

TABLE II

cephalexin	8	64	8	16	8
	8	8	0.125	8	64
	32	32	1	8	32
CH_2-C≡CH	32	32	2	4	8
CH_2CO_2Me	128	128	4	8	8
CH_2CH_2SMe	128	128	4	16	64
CH_2CH_2-N-phthalimide	8	32	4	8	128
	32	32	0.5	8	128
	128	128	4	32	128

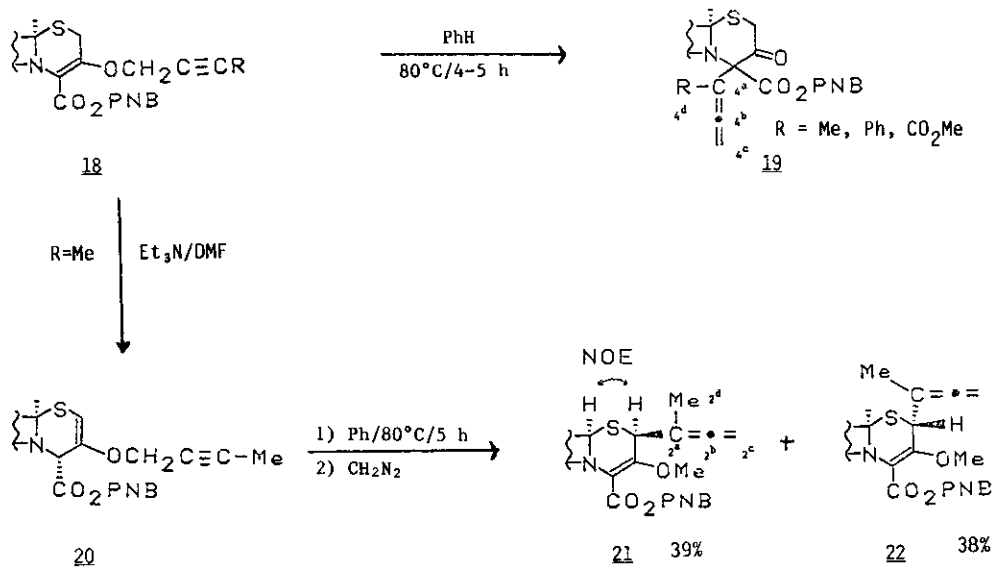
We then turned our attention from alkyl ethers to C(3)-ethers having some sort of functional group in order to attempt to increase the antibacterial activity, for example, substituted benzyls, unsaturated groups (allyl and propargyl), esters, a serine protected ester, sulfides, imides, terpenes, and nucleosides (see Table II).

Stability problems were encountered with allyl ethers and these were shown to readily undergo the Claisen rearrangement (80°C/PhH/1 h) to give a single product. Only one isomer was detected at C(4). The Claisen product 17 (R'=H) was identical (tlc, nmr) to the C-alkylated by-product of 3 using allyl alcohol.



16 17

Propargyl cephem ethers also undergo the Claisen rearrangement to give allenes 19, but require longer reaction times (4-5 h/80°C). Again only one isomer was detected at C(4). These are rather mild conditions for a propargyl Claisen rearrangement and we attributed this to the electronic effect of the β-carboalkoxy group, however, the Δ^2-cephem propargyl ether 20 also rearranges under similar conditions to give a C(2)-mixture of allenes 21 and 22. The stereochemistry at C(2) was determined by H^2-H^6 NOE studies and the presence of H^2-H^7 five bond coupling[10,11] (J=0.5 Hz) in 22 which was absent in 21.



In conclusion, we have shown the viability of the Mitsunobu reaction in preparing C(3)-alkoxycephems. Several of these derivatives show good in vitro antibacterial activity when compared to cephalexin.

EXPERIMENTAL

All melting points are uncorrected. The following instruments were used for obtaining the spectral data: ^{1}H nmr: Varian T-60, JEOL FX-90Q, Bruker WM-270 and 360: ^{13}C nmr: Bruker-270, JEOL FX-90Q; ir spectra: Perkin-Elmer 281; mass spectral data: Varian-m.a.t.-731.

(7β)-3-Methoxy-7-[(phenoxyacetyl)amino]-3-cephem-4-carboxylic Acid, (4-nitrophenyl)-methyl Ester 5 (R=Me)

To a stirred solution of 3 (0.385 g, 1 mM) in 20 ml of THF was added 1 equiv. Ph_3P (0.262 g, 1 mM), 1 equiv. MeOH (0.032 g, 1 mM) followed by the addition of 1 equiv. (0.146 g) dimethyl azodicarboxylate using 5 ml of THF as washing. Allowed to stir at r.t. for 20 min. Evaporated to a low volume, added 30 ml of EtOAc and washed 4x 10 ml H_2O, 1x brine, dried (Na_2SO_4) and evaporated to dryness. Chromatographed on silica gel using a toluene-ethyl acetate gradient to give:

Frac. 14-17, 67 mg (13%) C-alkylated product 4 (R=Me) as a froth; m/e 499; ir ν ($CHCl_3$) 1775 cm^{-1}(β-lactam), 1728 cm^{-1}(ketone); nmr ($CDCl_3$)δ 2.00 (s, 3, Me), 3.03, 3.70 (AB, J= 14 Hz, 2, C(2) protons), 4.63 (s, 2, $PhOCH_2$), 5.33-5.43 (BM, 4, H^6 H^7, PNB).

Frac. 18-22, 356 mg (71%) O-alkylated product 5 (R=Me) as a white solid; m/e 499, ir ν ($CHCl_3$) 1773 cm^{-1}(β-lactam); nmr ($CDCl_3$-$DMSOd_6$)δ 3.70 (s, 2, C(2) protons), 3.88 (s, 3, OMe), 4.63 (s, 2, $PhOCH_2$) 5.12 (d, J= 4 Hz, 1, H^6), 5.33 (BS, 2, PNB), 5.47 (d,d J=4, 8 Hz, 1, H^7), 8.97 (d, J=8 Hz, 1, NH).

(7β)-3-(1-propenyloxy)-7-[(phenoxyacetyl)amino]-3-cephem-4-carboxylic Acid, (4-nitrophenyl)-methyl Ester 16 (R'=H)

To a solution of 0.800 g of the acetic acid solvate of 3 in 30 ml THF was added 1.1 equiv. (0.423 g) Ph_3P, 1.1 equiv. (0.094 g) of allyl alcohol followed by 1.1 equiv. (0.236 g) dimethyl azodicarboxylate. After 15 min at r.t. the mixture was worked (vide supra) and chromatographed on silica gel using a toluene-ethyl acetate gradient to give:

Frac. 24-27, 144 mg (19%) C-alkylated product 4 (R=$CH_2CH=CH_2$) [17, R'=H] as a froth; m/e 525; ir ν ($CHCl_3$) 1775 cm^{-1} (β-lactam), 1728 cm^{-1} (ketone); nmr ($CDCl_3$) δ 3.00, 3.65 (AB, J = 15 Hz, 2, C(2) protons), 3.12 (m, 2, $\underline{CH_2}$-$CH=CH_2$), 4.60 (s, 2, $PhOCH_2$), 4.90-6.00 (m, 7, PNB, $CH=CH_2$, H^6, H^7).

F 29-34, 525 mg (57%) O-alkylated product 5 (R=$CH_2CH=CH_2$) [16, R'=H] as a froth; m/e 525; ir ν ($CHCl_3$) 1775 cm^{-1} (β-lactam), nmr ($CDCl_3$) δ 3.40 (s, 2, C(2) protons), 4.58 (s, 2, $PhOCH_2$), 5.10 (d J = 4 Hz, 1, H_6), 5.37 (s, 2, PNB), 5.5-6.2 (m, 4, H_6 + $CH=CH_2$).

A solution of 16 (R'=H) (578 mg) in 30 ml of PhH was gently refluxed 1 hour and evaporated to 17 (R'=H). TLC and nmr show complete conversion of 16 to 17.

(7β)-3-(2-Butynyloxy)-7-[(phenoxyacetyl)amino]-3-cephem-4-carboxylic Acid, (4-nitrophenyl)-methyl Ester 18 (R=Me)

To a solution of 1.00 g of the acetic acid solvate of 3 in 40 ml of THF was added 1.1 equiv. (0.529 g) Ph_3P, 1.1 equiv. (0.141 g) 2-butyn-1-ol followed by 1.1 equiv. (0.295 g) dimethyl azodicarboxylate. After 15 min at r.t. the mixture was worked up (vide supra) and chromatographed on silica gel using a toluene-ethyl acetate gradient to give:

Frac. 21-23, 71 mg (7%) C-alkylated product 4 (R=$CH_2C{\equiv}CCH_3$) as a white froth; m/e 537; ir ν ($CHCl_3$) 1775 cm^{-1} (β-lactam), 1729 cm^{-1} (ketone); nmr ($CDCl_3$) δ 1.70 (m, 3, Me), 3.03, 3.52 (AB, J=16 Hz, 2, C(2) protons), 3.32 (m, 2, $\underline{CH_2}C{\equiv}CCH_3$), 4.58 (s, 2, $PhOCH_2$), 3.45 (m, 4, PNB, H^6, H^7).

Frac. 31-70, 566 mg (57%) O-alkylated product 18 (R=Me) as a white froth; m/e 537; ir ν ($CHCl_3$) 2250 cm^{-1} (C≡C), 1778 cm^{-1} (β-lactam); nmr ($CDCl_3$), δ 1.87 (m, 3, Me), 3.50 (s, 2, C(2) protons), 4.60 (s, 2, $PhOCH_2$), 4.72 (m, 2, $O\underline{CH_2}C{\equiv}CCH_3$), 5.10 (d, J=4 Hz, 1, H^6), 5.33 (m, 2, PNB), 5.67 (d, d J=4, 8 Hz, 1, H^7). Crystallized from CH_2Cl_2-hexanes to give white needles, m.p. 97-120°C (undergoing Claisen as indicated by tlc). Anal. Calcd for $C_{26}H_{23}N_3O_8S$: C, 58.09; H, 4.31; N, 7.82; Found: C, 57.89; H, 4.01; N, 7.54.

(7β)-4-(1-Methyl-1,2-propadienyl)-3-oxy-7-[(phenoxyacetyl)amino]cephem-4-carboxylic Acid, (4-nitrophenyl) methyl Ester 19 (R=Me)

A solution of 18 (R=Me) (0.120 g) in 30 ml of PhH was gently refluxed for 3 h and evaporated to give 19 (R=Me) as a froth; m/z 537; ir ν ($CHCl_3$) 1950 cm^{-1} (allene), 1778 cm^{-1} (β-lactam), 1720 cm^{-1} (ketone); ^1H nmr ($CDCl_3$) δ 1.82 (m, J=3.0 Hz, 3, Me), 3.48 (s, 2, C(2) protons), 4.57 (s, 2, $PhOCH_2$), 5.01 (m, J=3.0 Hz, 2 allene methylene), 5.27, 5.43 (AB, 2, PNB), 5.31 (d, J=5.0 Hz, 1, H^6), 5.53 (d, d J=5, 8 Hz, 1, H^7); ^{13}C nmr ($CDCl_3$) δ C^2 33.63 t, C^3 191.87 s, C^4 73.58 s, C^{4a} 95.70 s, C^{4b} 207.59 s, C^{4c} 79.29 t, C^{4d} 14.98 q, C^6 57.79 d, C^7 58.51 d.

(4α,7β)-3-(2-Butynyloxy)-7-[(phenoxyacetyl)amino]-2-cephem-4-carboxylic Acid, (4-nitrophenyl)-methyl Ester 20

1.725 g of 18 (R=Me) in 25 ml of DMF was treated at r.t. for 23 h with 3.0 equiv. (1.34 ml) Et_3N. EtOAc was then added and then washed 1X 1NHCl, 4X H_2O, 1X brine, dried (Na_2SO_4), evaporated to dryness and chromatographed on silica gel using toluene-ethyl acetate gradient to give a 0.546 g (32%) 20 as a white solid; *ir* ν (*$CHCl_3$*) 1774 cm^{-1} (*β-lactam*); *nmr* (*$CDCl_3$*) δ 1.87 (*m*, 3, *Me*), 4.37 (m, 2, $O\underline{CH_2}C{\equiv}CMe$), 4.53 (s, 2, $PhO\underline{CH_2}$), 5.00 (bs, 1, H^4), 5.35 (m, 3, PNB, H^6), 5.70 (d, d J=4, 9 Hz, 1, H^7).

(2α or 2β,7β)-3-Methoxy-2-(1-methyl-1,2-propadienyl)-7-[(phenoxyacetyl)amino]-3-cephem -4-carboxylic Acid, (4-nitrophenyl)methyl Ester 21 and 22

20 (0.682 g) in 80 ml of PhH was gently refluxed for 5 h. Cooled to r.t. and evaporated to drynesss. CH_2Cl_2 (50 ml) Was added and treated at 5°C with excess CH_2N_2 for 8 min. It was then evaporated to dryness and chromatographed on silica using a toluene-ethyl acetate gradient to give:

Frac. 17-21, 0.263 g (38%) 22 as a white froth; m/e 551; ir ν ($CHCl_3$) 1945 cm^{-1} (allene), 1770 cm^{-1} (β-lactam); ^1H nmr ($CDCl_3$) δ 1.88 (m, $J_{2^d-2^c}$ = 3.05 Hz, 3, Me), 3.75 (s, 3, OMe), 3.99 (m, $J_{H^2-2^c}$ = 2.44 Hz, $J_{H^2-H^7}$ = 0.5 Hz, 1, H_2), 4.56 (s, 2, $PhOCH_2$), 4.85 (m, $J_{2^c-2^d}$ = 3.05 Hz, $J_{2^c-H^2}$ = 2.44 Hz, 11.06 Hz, 2, allene CH_2), 5.06 (d, J=4.88 Hz, 1, H^6), 5.42, 5.30 (m, 2, PNB), 5.89 (d, d J=4.88, 9.16, 0.5 Hz, 1, H^7). NOE's were observed between H^2-2^d and H^2-C(3)-OMe but not H^2-H^6; ^{13}C nmr ($CDCl_3$) δ C^2 43.15, C^{2a} 98.81, C^{2b} 207.02, C^{2c} 78.46, C^{2d} 17.24, C^3 160.81.

Frac. 22-27, 0.279 g (39%) 21 as a white froth; m/z 551; ir ν ($CHCl_3$) 1953 cm^{-1} (allene), 1779 cm^{-1} (β-lactam); ^1H nmr ($CDCl_3$) δ 1.90 (m, $J_{2^d-2^c}$ = 2.4 Hz, 3, Me), 3.90 (s, 3, OMe), 4.06 (m, $J_{H^2-2^c}$ = 3.0 Hz, 1, H^2), 4.60 (s, 2, $PhOCH_2$), 4.98, 4.85 (m, $J_{H^2-2^c}$ = 3.0, 3.7 Hz, 2, allene CH_2), 5.25, 5.42 (m, 2, PNB), 5.34 (d, J=3.7 Hz, 1, H^6), 5.55 (d, d J= 3.7, 7.9, 1, H^7). NOE's were observed between H^2-H^6, and H^2-2^d; ^{13}C nmr ($CDCl_3$) δ C^2 45.23, C^{2a} 95.18, C^{2b} 204.67, C^{2c} 79.84, C^{2d} 16.74, C^3 160.75.

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6. We abbreviate dimethyl azodicarboxylate as MAD since DMAD is used for dimethyl acetylenedicarboxylate.

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8. The reaction of 3 with 90% $Me^{18}OH$ under MAD-CAT conditions gave 61% $Ph_3P^{18}O$ (80% incorporation), 54% 5 (R=Me) and 13% 4 (R=Me). Neither 4 or 5 contained ^{18}O.

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