SYNTHESIS AND ANTIFUNGAL ACTIVITIES OF ALANINE-EXCHANGED ANALOGS OF PRADIMICIN A

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A series of pradimicin analogs were designed and synthesized to investigate the effect of the amino acid side chain on the antifungal activity. The alanine-exchanged analogs (**3a**~**3q**) were synthesized from 4'-*N*-Cbz-pradimic acid by coupling with appropriate amino acids or their equivalents followed by deblocking. All the D-α-amino acid derivatives except D-proline analog, **3k** retained the antifungal activity.

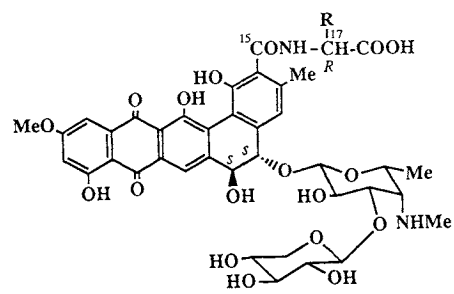
Pradimicin A (PRM A), is the original member of the pradimicin family of antibiotics produced by *Actinomadura hibisca* P157-2 (ATCC 53557)[1,2]. It is a broad-spectrum antifungal agent with demonstrable efficacy against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* infections in mice. Structurally, it is a glycosylated dihydrobenzo[*a*]naphthacenequinone having a D-alanine side chain at C-15. The D-alanine may be replaced by glycine (PRM D)[3] or D-serine (PRM FA-1)[4] without impairing antifugal activity, but the change to L-alanine resulted in a complete loss of antifungal activity, suggesting the importance of the amino acid side chain at C-15 in expression of antifungal activity[5]. The purpose of the present study was to design and synthesize a series of pradimicin analogs to investigate the effect of the amino acid side chain on the antifungal activity with a hope of identifying a derivative having an increased potency. This report describes the synthesis and antifungal activity of the alanine-exchanged analogs of PRM A.

Chemistry

Recently we have established a method of converting 4'-*N*-Cbz-PRM A into 4'-*N*-Cbz-pradimic acid (**1**)[6], an *N*-protected dealanyl-PRM A.

A series of pradimicin analogs were prepared from **1** by one of the following two general methods (A and B). Treatment of **1** with 1-hydroxybenzotriazole and dicyclohexylcarbodiimide (DCC) in THF gave an active ester, which was allowed to react with an appropriate amino acid or amino compound in 50% aqueous dioxane in the presence of triethylamine (Method A). Alternatively, the same active ester was reacted with an appropriate amino acid methyl ester in the presence of *N,O*-bis(trimethylsilyl)acetamide in THF followed by

Fig. 1. Structures of natural pradimicins.



Pradimicin A R=CH_3
Pradimicin D R=H
Pradimicin FA-1 R=CH_2OH

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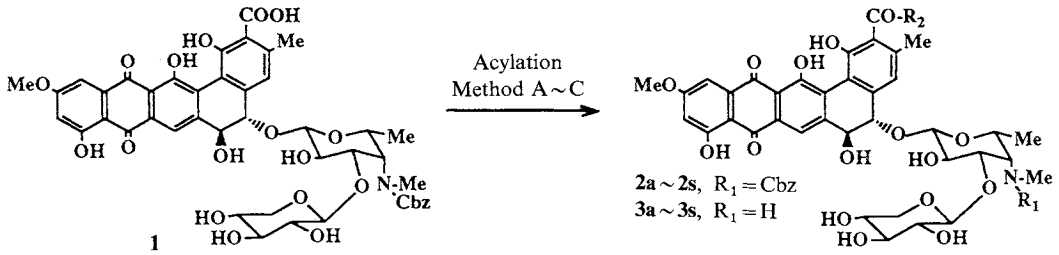
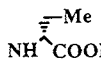
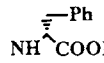
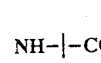
Table 1. Structures of amino acid analogs of pradimicin A.



1

Product	R_2	Method	Reagent	Yield
3a		B	D-Abu-OMe[a]	34%
3b		A	D-Phe	16%
3c		A	$H_2NC(CH_3)_2COOH$	19%
3d		B	D-Asp(OMe)-OMe	22%
3e		B	D-A_2pr(Cbz)-OMe[b]	20%
3f		A	D-Lys(Cbz)	13%
3g		A	D-Arg(NO_2)	13%
3h		A	L-Asp	29%
3i		B	L-Ser-OMe	26%
3j		A	L-A_2pr(Cbz)[b]	17%
3k		A	D-Pro	31%
3l		A	sarcosine	10%
3m		A	aminomethyltetrazole[c]	14%
3n		A	$NH_2CH_2SO_3H$	10%
3o		B	β-Ala-OMe	16%
3p		A	$NH_2CH_2CH_2NH_2$	25%
3q		C	$HOCH_2COOMe$	4%
3r	NH_2	—	—	81%[d]
3s	OH	—	—	70%[d]

[a] D-Abu-OMe = D-aminobutyric acid methyl ester, [b] A_2pr(Cbz) = N^3-Cbz-diaminopropionic acid, [c] Aminomethyltetrazole was prepared by a method of Ref 8, [d] **3r** and **3s** were prepared by catalytic hydrogenation of 4′-*N*-Cbz-pradimic acid amide[6], and 4′-*N*-Cbz-pradimic acid[6], respectively.

alkaline treatment (Method B). In both cases, the *N*-protecting group was removed by catalytic hydrogenation. The stereochemistries at C-17 were ascertained by HPLC comparison of pairs of the C-17 epimers, PRM FA-1 and **3i**, **3d** and **3h**, and **3e** and **3j**. The glycolate analog (**3q**) was prepared from **1** by esterification with glycolic acid in the presence of DCC and subsequent catalytic hydrogenation (Method C). Pradimic acid (**3s**) and its amide (**3r**) were prepared by catalytic hydrogenation of 4′-*N*-Cbz-pradimic acid (**1**) and its amide[6], respectively. The structures of the analogs, **3a**～**3s** and the methods of preparation are listed in Table 1.

Antifungal Activity

In Vitro Activity

Table 2 summarizes the MICs of the derivatives synthesized in this study determined by the 2-fold agar dilution method on yeast morphology agar buffered with 0.067 M phosphate (pH 7.0). The naturally occurring PRMs A, FA-1 and D were equal in the potency and spectrum of activity. However, among the C-17-substituted D-α-amino acid derivatives synthesized, the activity changed depending on the nature of substituent at C-17. The D-α-aminobutyric acid analog (**3a**) was 2-fold less active than PRM A against *C. albicans* and *A. fumigatus*. The D-phenylalanine analog (**3b**) showed good activity against *C. neoformans* and *A. fumigatus*, but not active against *C. albicans* at 100 μg/ml. The D-aspartic acid analog (**3d**) showed rather weak activity against *A. fumigatus*. The D-diaminopropionic acid analog (**3e**) was almost as active as PRM A against *C. neoformans* and *A. fumigatus*. In general, introduction of a neutral or basic group to the C-17-position did not appear to alter the activity against *C. neoformans* and *A. fumigatus*, while substitution with an acidic substituent reduced the activity against *A. fumigatus*. When compared the activities of the D- and L-amino acid analogs, most of the L-isomers, **3h** and **3j** were found inactive as the L-alanine analog of PRM A. Only the L-serine isomer, **3i** had an MIC of 25 μg/ml against *C. albicans*. The experiment comparing **3i** and PRM FA-1 indicated that **3i** was fungistatic against *C. albicans* in YNG (Fig. 2). The C-17-dimethyl analog (**3c**) is only active against yeasts. The compounds having a substituent on *N*-16 (**3k** and **3l**) showed no activity, suggesting that the amide proton is essential for the antifungal activity. Pradimic acid amide, **3r** and pradimic acid, **3s** showed no antifungal activity. The glycolic acid analog (**3q**), ethylenediamine analog (**3p**), and aminomethanesulfonic acid analog (**3n**) showed no activity. The aminomethyltetrazole analog (**3m**) retained *in vitro* activity.

Table 2. *In vitro* activity of pradimicin derivatives.

Compound	MIC (μg/ml)[a]		
	Candida albicans A9540	*Cryptococcus neoformans* IAM 4514	*Aspergillus fumigatus* IAM 2034
3a	25	3.1	6.3
3b	>100	1.6	6.3
3c	12.5	6.3	>100
3d	12.5	6.3	25
3e	25	1.6	3.1
3f	25	6.3	6.3
3g	>100	6.3	3.1
3h	>100	>100	>100
3i	25	25	>100
3j	>100	>100	>100
3k	>100	>100	>100
3l	>100	>100	>100
3m	6.3	3.1	3.1
3n	>50	>50	>50
3o	>100	>100	>100
3p	>100	>100	>100
3q	>100	>100	>100
3r	>100	>100	>100
3s	>100	>100	>100
PRM A	12.5	0.8	3.1
PRM D	6.3	0.8	6.3
PRM FA-1	12.5	0.8	6.3

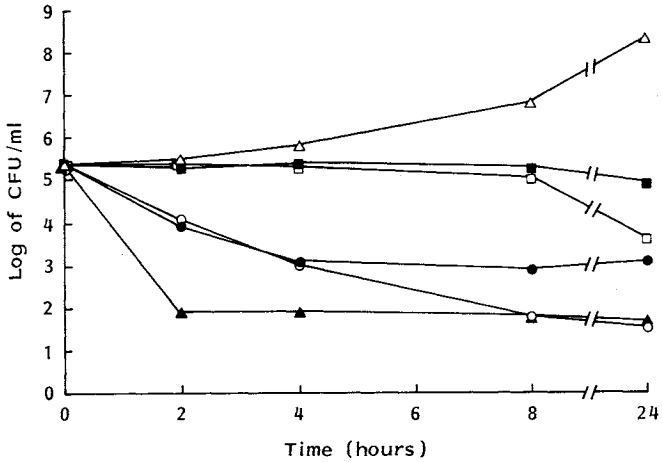
[a] MICs were determined by the two-fold agar dilution method on yeast morphology at pH 7.0 (Incubation, 28°C, 48 hours).

In Vivo Activity

The *in vivo* activities of compounds, **3d** and **3e**

Fig. 2. Effects of pradimicins FA-1 and 17-*epi*-FA-1 on growing cells of *Candida albicans* A9540.

Cells of *C. albicans* A9540 (initial inoculum: 2.5×10^5 cfu/ml) were incubated at 28°C with shaking (100 rpm) in YNG, pH 7.0, in the presence of MIC (6.3 μg/ml, ● and 25 μg/ml, ○) of pradimicin FA-1, MIC (25 μg/ml, ■ and 100 μg/ml, □) of 17-epipradimicin FA-1, and amphotericin B (1.6 μg/ml, ▲). △, Control.



were evaluated against a systemic *C. albicans* infection in mice and compared with those of PRMs A, D and FA-1. The kidney is the target organ of systemic *C. albicans* infection in mice where *C. albicans* continues to multiply, leading to renal failure. Table 3 summarizes the results showing that the diaminopropionic acid analog (**3e**) was as efficacious as natural PRMs. The results indicated poor correlation of *in vitro-in vivo* activities.

Table 3. *In vitro* and *in vivo* activities of pradimicin derivatives.

Compound	MIC (μg/ml)[a] *Candida albicans* A9540	PD_{50} (mg/kg, iv)[b] *Candida albicans* A9540
3d	12.5	27
3e	25	.10
PRM A	12.5	8.9
PRM D	6.3	9.0
PRM FA-1	12.5	18

[a] MICs were determined by the two-fold agar dilution method on yeast morphology at pH 7.0 (Incubation, 28°C, 48 hours).

[b] PD_{50} value was determined 20 days after the fungal challenge.

Experimental

MPs were determined using a Yanagimoto micro hot-stage apparatus and are uncorrected. NMR spectra were recorded on a JEOL GX-400 (400 MHz), and mass spectra on a JEOL JMS-AX505H (FAB) mass spectrometer.

Method A

D-Phenylalanine Analog of Pradimicin A (**3b**)

A mixture of 4′-*N*-Cbz-pradimic acid (**1**) (50 mg, 0.055 mmol), 1-hydroxybenzotriazole (10 mg, 0.065 mmol) and DCC (14 mg, 0.068 mmol) in 1 ml of tetrahydrofuran was stirred for 1 hour at room temperature. The dicyclohexylurea formed was removed by filtration and the filtrate was concentrated. To the residue was added D-phenylalanine (18 mg, 0.11 mmol) and triethylamine (38 μl, 10.27 mmol) in 1 ml of dioxane - H_2O (1 : 1). The mixture was stirred overnight at room temperature and then concentrated to volume of 2 ml. The mixture was acidified with 1 N HCl to pH 2. The precipitate was collected by filtration and the product was chromatographed on a C_{18} silica gel column (Bondapak C_{18}, 20 i.d. × 200 mm) eluting with 40 ~ 45% CH_3CN - 0.001 N HCl. The fractions containing the desired compound were combined

Table 4. ^{1}H NMR and MS spectral data of **3**.

No.	^{1}H NMR δ (400 MHz, DMSO-d_6) ppm	FAB-MS m/z $(M+H)^+$	MP
3a	0.96 (3H, t, J=7 Hz, CH_2CH_3), 1.28 (3H, d, J=7 Hz, 5'-CH_3), 1.6~1.8 (2H, m, CH_2CH_3), 2.32 (3H, s, 3-CH_3), 4.30 (1H, ddd, J=5, 7, 9 Hz, CH), 4.48 (1H, d, J=6 Hz, 1''-H), 4.79 (1H, br, 1'-H), 8.45 (1H, d, J=7 Hz, CONH)	855	>220°C (grad. dec.)
3b	1.27 (3H, d, J=6 Hz, 5'-CH_3), 2.05 (3H, s, 3-CH_3), 4.47 (1H, d, J=7 Hz, 1''-H), 4.64 (1H, m, CH), 4.77 (1H, br, 1'-H), 7.2~7.4 (5H, m, phenyl), 8.58 (1H, d, J=6 Hz, CONH)	917	>210°C (grad. dec.)
3c	1.28 (3H, d, J=6 Hz, 5'-CH_3), 1.43 (3H, s, $C(CH_3)_2$), 1.44 (3H, s, $C(CH_3)_2$), 2.32 (3H, s, 3-CH_3), 4.47 (1H, d, J=6 Hz, 1''-H), 4.79 (1H, br, 1'-H), 8.47 (1H, s, CONH)	855	>210°C (grad. dec.)
3d	1.28 (3H, d, J=7 Hz, 5'-CH_3), 2.30 (3H, s, 3-CH_3), 2.67 (1H, m, CH_2), 2.81 (1H, dd, J=7, 17 Hz, CH_2), 4.48 (1H, d, J=7 Hz, 1''-H), 4.74 (1H, q, J=7 Hz, CH), 4.77 (1H, br, 1'-H), 8.52 (1H, d, J=8 Hz, CONH)	885	>210°C (grad. dec.)
3e	1.28 (3H, d, J=6 Hz, 5'-CH_3), 2.28 (3H, s, 3-CH_3), 4.47 (1H, d, J=7 Hz, 1''-H), 4.72 (1H, m, CH), 4.79 (1H, d, J=8 Hz, 1'-H), 8.74 (1H, d, J=9 Hz, CONH)	856	>210°C (grad. dec.)
3f	1.28 (3H, d, J=7 Hz, 5'-CH_3), 1.4~1.8 (6H, m, $(CH_2)_3$), 2.32 (3H, s, 3-CH_3), 2.74 (2H, m, CH_2), 4.37 (1H, m, CH), 4.50 (1H, d, J=6 Hz, 1''-H), 4.78 (1H, br, 1'-H), 8.50 (1H, d, J=8 Hz, CONH)	898	>200°C (grad. dec.)
3g	1.28 (3H, d, J=6 Hz, 5'-CH_3), 1.6~1.7 (4H, m, CH_2CH_2), 1.8~1.9 (2H, m, CH_2), 2.31 (3H, s, 3-CH_3), 4.39 (1H, m, CH), 4.46 (1H, d, J=7 Hz, 1''-H), 4.78 (1H, br, 1'-H), 8.55 (1H, d, J=7 Hz, CONH)	926	>190°C (grad. dec.)
3h	1.28 (3H, d, J=7 Hz, 5'-CH_3), 2.31 (3H, s, 3-CH_3), 2.6~2.7 (1H, m, CH_2), 2.82 (1H, dd, J=6, 16 Hz, CH_2), 4.47 (1H, d, J=7 Hz, 1''-H), 4.74 (1H, q, J=6 Hz, CH), 4.77 (1H, br, 1'-H), 8.57 (1H, d, J=7 Hz, CONH)	885	>230°C (grad. dec.)
3i	1.28 (3H, d, J=6 Hz, 5'-CH_3), 2.36 (3H, s, 3-CH_3), 3.7~3.8 (2H, m, CH_2), 4.45 (1H, m, CH), 4.78 (1H, br, 1'-H), 8.30 (1H, d, J=8 Hz, CONH)	857	>210°C (grad. dec.)
3j	1.28 (3H, d, J=7 Hz, 5'-CH_3), 2.34 (3H, s, 3-CH_3), 4.48 (1H, d, J=7 Hz, 1''-H), 4.69 (1H, m, CH), 4.70 (1H, br, 1'-H), 8.80 (1H, d, J=8 Hz, CONH)	856	>210°C (grad. dec.)
3k	1.28 (3H, d, J=7, 5'-CH_3), 1.8~2.0 (2H, m, CH_2), 2.29 (3H, s, 3-CH_3), 4.42 (1H, dd, J=4, 8, CH), 4.47 (1H, br, 1''-H), 4.80 (1H, br, 1'-H)	867	>200°C (grad. dec.)
3l	1.27 (3H, br, 5'-CH_3), 2.25 (3H, s, 3-CH_3), 4.48 (1H, d, J=6 Hz, 1''-H), 4.80 (1H, br, 1'-H)	841	>210°C (grad. dec.)
3m	1.28 (3H, d, J=7 Hz, 5'-CH_3), 2.24 (3H, s, 3-CH_3), 4.48 (1H, d, J=7 Hz, 1''-H), 4.69 (1H, dd, J=15, 6 Hz, CH_2), 4.74 (1H, dd, J=15, 6 Hz, CH_2), 4.78 (1H, br, 1'-H), 8.99 (1H, t, J=6 Hz, CONH)	851	>190°C (grad. dec.)
3n	1.29 (3H, d, J=6 Hz, 5'-CH_3), 2.35 (3H, s, 3-CH_3), 4.07 (1H, br, CH_2), 4.17 (1H, br, CH_2), 4.46 (1H, d, J=7 Hz, 1''-H), 4.81 (1H, br, 1'-H), 8.40 (1H, t, J=6 Hz, CONH)	863	>230°C (grad. dec.)
3o	1.28 (3H, d, J=6 Hz, 5'-CH_3), 2.26 (3H, s, 3-CH_3), 4.49 (1H, d, J=7 Hz, 1''-H), 4.77 (1H, br, 1'-H), 8.26 (1H, t, J=6 Hz, CONH)	841	>200°C (grad. dec.)
3p	1.23 (3H, d, J=7 Hz, 5'-CH_3), 2.28 (3H, s, 3-CH_3), 4.48 (1H, d, J=6 Hz, 1''-H), 4.78 (1H, br, 1'-H), 8.43 (1H, t, J=6 Hz, CONH)	812	>210°C (grad. dec.)
3q	1.28 (3H, d, J=6 Hz, 5'-CH_3), 2.37 (3H, s, 3-CH_3), 4.47 (1H, d, J=6 Hz, 1''-H)	828	>210°C (grad. dec.)
3r	1.24 (3H, d, J=7 Hz, 5'-CH_3), 2.27 (3H, s, 3-CH_3), 4.45 (1H, d, J=7 Hz, 1''-H), 4.72 (1H, d, J=7 Hz, 1'-H)	769	>190°C (grad. dec.)
3s	1.23 (3H, d, J=6 Hz, 5'-CH_3), 2.58 (3H, s, 3-CH_3), 4.43 (1H, d, J=8 Hz, 1''-H), 4.74 (1H, d, J=7 Hz, 1'-H)	770	>230°C (grad. dec.)

and concentrated to give 29 mg of D-phenylalanine analog of 4'-*N*-Cbz-pradimicin A (**2b**) (yield 50%).

A mixture of **2b** (27 mg, 0.026 mmol) and 10% Pd-C (14 mg) in MeOH (1.5 ml) - 1 N HCl (0.3 ml) was stirred under hydrogen for 4 hours at room temperature. The Pd-C was removed by filtration and the filtrate was concentrated to *ca.* 1 ml. The concentrate was chromatographed on a C_{18} silica gel column

(Bondapak C_{18}, 20 i.d. × 200 mm) eluting with 30~40% CH_3CN-H_2O. The desired fractions were combined, concentrated and freeze dried to give 7.3 mg (yield 31%) of 2-D-phenylalanine analog of pradimicin A (**3b**).

According to Method A, compounds **3c**, **3f**~**3p** (except **3i** and **3o**) were prepared from 4'-*N*-Cbz-pradimic acid (**1**). Their spectra data are summarized in Table 4.

Method B

D-2-Aminobutyric Acid Analog of Pradimicin A (3a)

A mixture of 4'-*N*-Cbz-pradimic acid (**1**) (82 mg, 0.091 mmol), 1-hydroxybenzotriazole (17 mg, 0.11 mmol) and DCC (22 mg, 0.11 mmol) in tetrahydrofuran (1.5 ml) was stirred for 1 hour at room temperature. The dicyclohexylurea was removed by filtration and the filtrate was concentrated. To the active ester was added D-2-aminobutyric acid methyl ester hydrochloride (42 mg, 0.27 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (290 ml, 1.7 mmol) in tetrahydrofuran (0.5 ml). The mixture was stirred for 3 days at room temperature. An additional BSA (290 ml, 1.7 mmol) and D-2-aminobutyric acid methyl ester (42 mg, 0.27 mmol) were then added to the solution and the stirring was continued for further 3 days. The THF was removed *in vacuo* and to the residue was added 1 N HCl (5 ml) and MeOH (5 ml) to deblock trimethylsilyl ether. After 1 hour of stirring, the mixture was cooled in ice bath and 1 N NaOH (7 ml) was added to hydrolyze the methyl ester. MeOH was removed by evaporation and the residue was acidified with 3 N HCl. The precipitate formed was collected by filtration and was purified by column chromatography (Bondapak C_{18}, 20 i.d. × 200 mm) using 30~40% CH_3CN-pH 3.5 buffer as eluent to give 43 mg of **2a** (yield 48%).

A mixture of **2a** (40 mg, 0.04 mmol) and 10% Pd-C (12 mg) in 4.5 ml of MeOH-1 N HCl (10:1) was hydrogenated for 1 hour at room temperature. After MeOH was removed, the residue was chromatographed on a C_{18} silica gel column (Bondapak C_{18}, 20 i.d. × 200 mm) eluting with 30% CH_3CN-0.001 N HCl. The appropriate fractions were combined, concentrated, and lyophilized to give the D-2-aminobutyric acid analog of pradimicin A (**3a**) (24.6 mg, yield 71%).

According to Method B, compounds (**3d**), (**3e**), (**3i**), (**3o**) were prepared from 4'-*N*-Cbz-pradimic acid (**1**). Their spectra data are summarized in Table 4.

Method C

Glycolic Acid Analog of Pradimicin A (3q)

A mixture of **1** (101 mg, 0.11 mmol), DCC (46 mg, 0.22 mmol) and methyl glycolate (112 μl, 1.45 mmol) in THF was stirred overnight. The dicyclohexylurea formed was removed by filtration and the filtrate was concentrated. The residue was dissolved in 4 ml of 2 N NaOH and the solution was stirred for 1 hour. After acidification with 3 N HCl, the precipitate was collected by filtration and chromatographed on a C_{18} silica gel column (Bondapak C_{18}, 20 i.d. × 200 mm) using 35~40% CH_3CN-0.001 N HCl as eluent to give 24 mg (yield 23%) of **2q**.

A mixture of **2q** (21 mg, 0.04 mmol) and 10% Pd-C (10 mg) in 2.2 ml of MeOH-1 N HCl (10:1) was hydrogenated for 1 hour at room temperature. The MeOH was removed and the mixture was chromatographed on a C_{18} silica gel (Bondapak C_{18}, 20 i.d. × 200 mm) using 30~40% CH_3CN-0.001 N HCl as eluent. The fractions containing the desired compound were combined, concentrated, and lyophilized to give the glycolic acid analog of pradimicin A (**3q**) (3 mg, yield 17%).

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