Synthesis and Biological Evaluation of *N*-Formyl Hydroxylamine Derivatives as Potent Peptide Deformylase Inhibitors

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Antibacterial resistance to hospital-acquired Gram-positive bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE), has been increasing at an alarming rate. Therefore there is an urgent need to identify novel antibacterial drugs with new modes of action. Peptide deformylase (PDF), which catalyzes the removal of the *N*-terminal formyl group from newly synthesized polypeptides and is essential in a variety of pathogenic bacteria but is not required for cytoplasmic protein synthesis in eukaryotes, is an attractive target for the discovery of novel antibiotics.[1] Many PDF inhibitors have been reported in recent years, some of which showed excellent antibacterial activity *in vitro* and in animal models. Amongst these inhibitors, NVP-PDF-713 (**2**, Vicuron and Novartis)[2] and BB-83698 (**3**, Vernalis)[3] are currently in phase I clinical trials for the treatment of respiratory tract infections (Fig. 1). Based on the mechanism of deformylation of peptides and the pharmacophore requirements for PDF inhibitor, a new series of arylamidopiperidine derivatives at the P3' position was designed and synthesized. We chose *tert*-leucine as the P2' residue because other PDF inhibitors containing this P2' residue have shown excellent antibacterial activity and bioavailability.[4] We report here the synthesis and preliminary *in vitro* evaluation of a new series of PDF inhibitors having an arylamidopiperidine at P3' position.

The general route for the synthesis of the arylamidopiperidine derivatives is outlined in Scheme 1. The 1-Boc-4-aminopiperidine **6** was prepared in two steps from commercially available *N*-Boc-piperidin-4-one **5** by reductive amination with benzyl amine followed by hydrogenolysis of the benzyl group. Acylation of the 4-amino group was readily accomplished by treating **6** with *p*-substituted benzoyl chloride to yield intermediate **7**. The piperidine hydrochloride **7** was subsequently coupled by standard peptide coupling conditions using *N*-Boc-L-*tert*-leucine to give amide **8**. Removal of the *N*-Boc group with HCl (g)/EtOAc gave amine hydrochloride **9**.

The *N*-formyl hydroxylamine analogues were synthesized as shown in Scheme 2. The required chiral intermediate **11** containing the P1' residue was prepared by using Evan's asymmetric alkylation methodology described previously literature.[5]

Reaction of (*S*)-4-benzyl-2-oxazolidinone with acid **10** in the presence of *n*-BuLi provided the corresponding oxazolidinone amide. Trichlorotitanium enolate of oxazolidinone amide in CH_2Cl_2 was obtained by treatment of the amide successively with 1.1 equiv each of $TiCl_4$ and Et_3N. Subsequent treatment of this enolate with 2.0 equiv of benzyl chloromethyl ether provided **11** selectively in 90% yield.[6] Removal of the auxiliary group of **11** by hydrolysis under

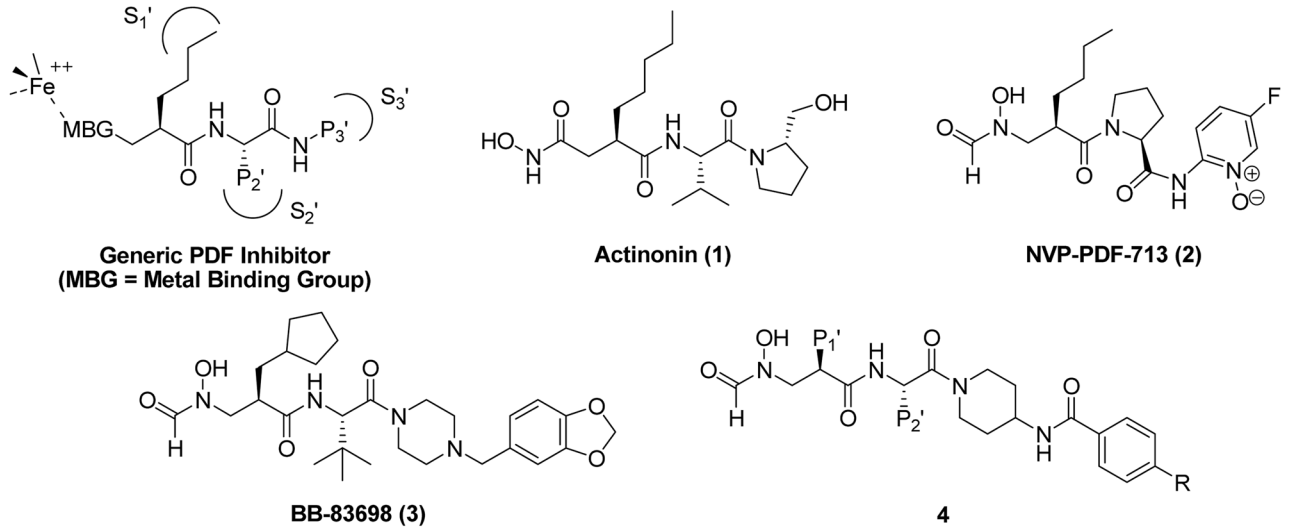
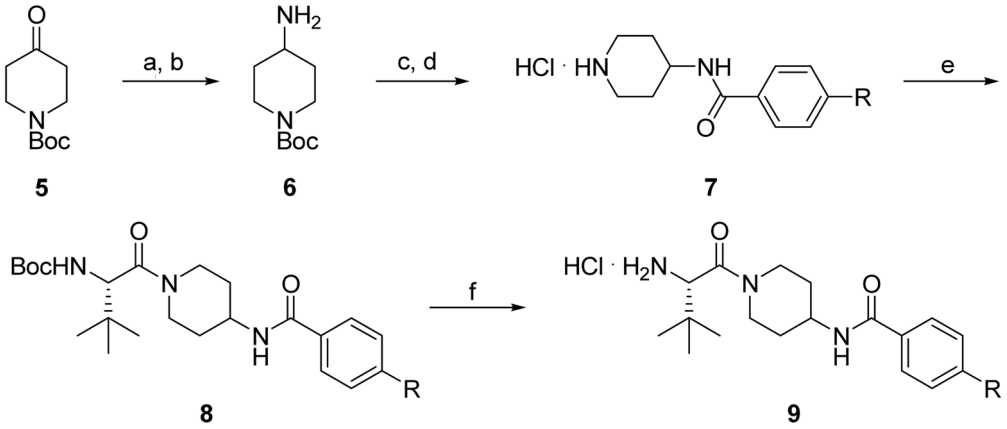


Figure 1. Chemical structure of generic PDF inhibitor, Actinonin (**1**), NVP-PDF-713 (**2**), BB-83698 (**3**) and the new series of arylamidopiperidine derivatives (**4**).

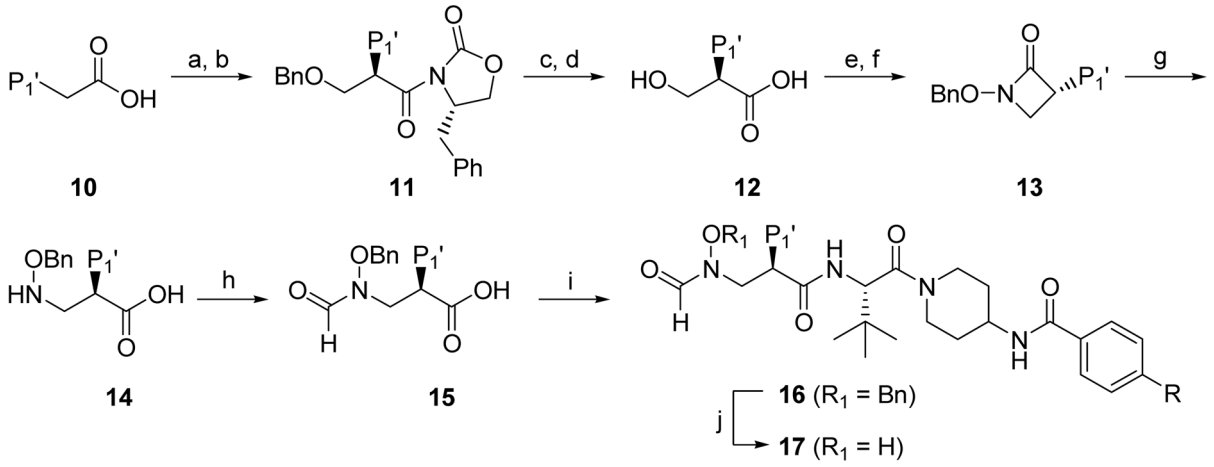


Scheme 1. Reagents and conditions: (a) benzylamine, $NaBH_3CN$, MeOH; (b) H_2, Pd/C, MeOH; (c) *p*-substituted benzoyl chloride, TEA, CH_2Cl_2; (d) HCl (g), EtOAc; (e) *N*-Boc-L-*tert*-leucine, EDCI, DMAP, CH_2Cl_2; (f) HCl (g), EtOAc.

$LiOH/H_2O_2$ conditions followed by catalytic hydrogenation gave alcohol **12**. Condensation of **12** with *O*-benzylhydroxylamine gave the benzyl hydroxamate, which was converted into β-lactam **13** through an intramolecular Mitsunobu reaction.[7] Hydrolysis of the β-lactam furnished acid **14**, which was subsequently formylated using acetic anhydride/formic acid[8] at its benzyloxyamine moiety to give the formamido acid **15**. Subsequent peptide coupling conditions of amine **9** to carboxylic acid **15** gave compound **16**. Removal of *O*-benzyl protecting group by Pd-catalysed hydrogenation yielded the *N*-formyl hydroxylamine **17**.

The activities of the compounds compared to actinonin **1** are summarized in Table 1. The activity of these inhibitors is strongly influenced by the nature of the P1' position (*e.g.*, **17c**, **17h**, **17i**) in the case of *E. coli* PDF. Derivative **17e**, with a cyclopentylmethyl substituent at the P1' position, was one of the most potent inhibitor of *E. coli* PDF enzyme with an IC_{50} value of 4 nM, and 8 times more active than that of *S. aureus* PDF. However, the enzyme also accommodated a variety of other substituents. Analogues with small lipophilic groups (F, CF_3) of *p*-substituted arylamidopiperidine were crucial for activity.

The antibacterial spectrum of these compounds compared to Linezolid and Vancomycin is presented in Table 2. Antibacterial activity against *S. pneumoniae* and *S. pyogenes* was observed for most of compounds. However, none of the tested compounds showed any activity against Gram-negative organism such as *Escherichia coli* and *Pseudomonas aeruginosa* (MIC >50 μg/mL). Compound **17a** showed the best improvement of antibacterial activity against these selected strains. Compounds having cycloalkyl (cyclopentylmethyl) at the P1' side chains showed more potent antibacterial activity than expected (**17a**, **17c**, **17d**, **17e**, **17k** and **17l**). We propose this may be related to greater lipophilicity of the molecule, connected with better permeation of the bacterial membrane. However, there was not a good correlation between the IC_{50} values and the minimum inhibitory concentrations (MIC). Compound **17a**, with nitrile group at the *p*-position on the phenyl ring, despite the fact that it is a much less potent PDF inhibitor than **17k** (60 nM versus 5 nM) showed a similar MIC profile. These results reinforce the observation of Clements *et al.*,[4] that



Scheme 2. Reagents & conditions: (a) *n*-BuLi, THF; (b) i) $TiCl_4$, TEA, ii) $BnOCH_2Cl$, CH_2Cl_2; (c) H_2O_2, LiOH, THF/H_2O; (d) H_2, Pd/C, EtOH; (e) $BnONH_2$·HCl, DMAP, EDCI, CH_2Cl_2; (f) PPh_3, DEAD, THF; (g) LiOH, THF/H_2O/MeOH; (h) HCOOH, Ac_2O, CH_2Cl_2; (i) **9**, HOBT, EDCI, NMM, DMF; (j) H_2, Pd/C, EtOH

Table 1. PDF enzyme inhibitory activity of arylamidopiperidine derivatives

Compds	P1'	R	*E. coli* PDF. Ni IC_{50}[a]	*S. aureus* PDF. Ni IC_{50}	*H. influenzae* PDF. Ni IC_{50}
1			6	17	11
17a	Cyclopentylmethyl	CN	60	52	104
17b	*n*-Butyl	H	12	55	108
17c	Cyclopentylmethyl	F	51	50	117
17d	Cyclopentylmethyl	H	5	73	> 200
17e	Cyclopentylmethyl	CF_3	4	31	ND
17f	Isobutyl	H	ND	> 200	58
17g	Isobutyl	CF_3	5	> 200	45
17h	*n*-Butyl	F	6	164	167
17i	Isobutyl	F	6	111	42
17j	*n*-Butyl	CH_3	52	112	186
17k	Cyclopentylmethyl	CH_3	5	28	> 200
17l	Cyclopentylmethyl	OCH_3	> 200	NT	NT

[a]IC_{50} (nM). ND: Not determined; NT: Not tested.

variations of antibacterial activity cannot necessarily be attributed to a corresponding variation in enzyme inhibition.

The precise reason remains to be investigated, but we can speculate that there might be several possible factors, such as poor penetration of the bacterial cell membrane, or recognition by an efficient efflux system of the bacteria.

In conclusion, a series of arylamidopiperidine analogues containing modifications to the P3' side chain have been synthesized and were tested in the PDF. We demonstrated that the antibacterial activity of this series might be maintained and improved with appropriate structural modifications. Further evaluations of inhibitors (**17a**, **17c** and **17k**) with regard to its selectivity and *in vivo* efficacy as well as SAR studies of this series of compounds are already underway in our laboratories.

Experimental Section

General. NMR spectra were recorded on a Bruker Avance II 400 (100.63 MHz for ^{13}C and 400.13 MHz for ^{1}H). Chemical shifts are indicated in δ values (ppm) downfield from internal TMS, and coupling constants (J) are given in Hertz (Hz). IR spectra were recorded on a Shimadzu IR 470 spectrophotometer. Mass spectra were recorded on an Agilent 1100 LC/MSD. The products were analyzed with a Zorbax XBD-C18 column (2.1 × 100 mm, 3.5 μm particles) using the linear gradient condition. The flow rate was 0.3 mL/min, and the eluent was monitored at 254 and 233 nm. The mass spectral mode of operation was positive ion electrospray (API-ES).

4-Amino-piperidine-1-carboxylic acid *tert*-butyl ester (6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.05 (br s, 2H), 2.85-2.70 (m, 3H), 1.80-1.77 (m, 2H), 1.45 (s, 9H), 1.31-1.20 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 154.8, 79.3, 51.2, 48.8, 35.5, 28.4; IR (KBr): 3352, 2970, 2920, 2850, 2210, 1585, 1559, 1422, 1361, 1339, 1250, 1167, 870, 765 cm^{-1}; LCMS (positive): 145.1 $(M\text{-}tBu+H)^+$.

4-Fluoro-*N*-piperidin-4-yl-benzamide HCl (7). ^{1}H NMR (400 MHz, CD_3OD): δ 7.95-7.90 (m, 2H), 7.23-7.17 (m, 2H), 4.22-4.14 (m, 1H), 3.52-3.49 (m, 2H), 3.21-3.13 (m, 2H), 2.22-2.18 (m, 2H), 1.99-1.89 (m, 2H); ^{13}C NMR (100.6 MHz, CD_3OD): δ 168.7, 165.0, 131.7, 131.1 131.0, 116.5, 116.2, 46.4, 44.3, 29.4; IR (KBr): 3494, 2972, 2825, 2505, 2455, 1912, 1638, 1597, 1539, 1498, 1389, 1339, 1316, 1222, 1155, 855 cm^{-1}.

{(S)-1-[4-(4-Fluro-benzoylamino)-piperidine-1-carbonyl]-2,2-dimethyl-propyl}-carbamic acid *tert*-butyl ester

Table 2. Minimum inhibitory concentrations of arylamidopiperidine derivatives

Compds	MIC (μg/mL) (organisms)[a]									
	S.au	MRSA[b]	MSSA[b]	VRE.fa[b]	VSE.fa[b]	VRE.f[b]	VSE.f[b]	PNSSP[b]	PSSP[b]	*S.py*
17a	1.6	3.1	3.1	3.1	3.1	1.6	1.6	1.6	1.6	0.1
17b	50	>50	12.5	25	25	25	6.3	0.8	3.1	1.6
17c	3.1	6.3	6.3	6.3	6.3	1.6	1.6	1.6	1.6	0.2
17d	ND	12.5	6.3	6.3	6.3	3.1	3.1	1.6	3.1	0.4
17e	ND	3.1	6.3	6.3	6.3	6.3	6.3	1.6	1.6	3.1
17f	>50	>50	>50	>50	>50	>50	>50	>50	>50	6.3
17g	50	>50	>50	>50	>50	>50	>50	25	25	6.3
17h	3.1	>50	>50	50	50	6.3	6.3	6.3	6.3	0.4
17i	>50	>50	>50	>50	>50	>50	>50	>50	>50	6.3
17j	50	>50	>50	>50	>50	3.1	25	ND	3.1	3.1
17k	1.6	6.3	3.1	3.1	3.1	0.8	1.6	1.6	1.6	3.1
17l	12.5	12.5	6.3	6.3	6.3	1.6	3.1	3.1	3.1	0.2
Linezolid	1.6	1.6	0.8	0.8	0.8	1.6	1.6	0.8	0.8	0.8
Vancomycin	0.8	3.1	6.3	>50	3.1	>50	1.6	0.8	0.8	0.8

[a]organisms: *S.au, Staphylococcus aureus* 503; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; VRE.fa, vancomycin-resistant *Enterococcus faecalis*; VSE.fa, vancomycin-susceptible *Enterococcus faecalis*; VRE.f, vancomycin-resistant *Enterococcus faecium*; VSE.f, vancomycin-susceptible *Enterococcus faecium*; PNSSP, penicillin-non susceptible *Streptococcus pneumoniae*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*; *S.py*, *Streptococcus pyogenes*.
[b]supplied from Yonsei University College of Medicine, Seoul, Korea.

(8). ^{1}H NMR ($CDCl_3$) δ 7.82-7.77 (m, 2H), 7.12-7.07 (m, 2H), 6.36-6.31 (t, 1H), 5.36-5.28 (m, 1H), 4.67-4.53 (m, 2H), 4.21-4.08 (m, 2H), 3.31-3.16 (m, 1H), 2.83-2.71 (m, 1H), 2.19-2.04 (m, 2H), 1.51-1.39 (m, 11H), 0.98-0.96 (d, 9H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.3, 165.8, 163.5, 155.7, 130.5, 129.3, 129.2, 115.6, 115.4, 79.5, 55.6, 47.2, 47.1, 45.9, 45.2, 41.1, 40.9, 35.8, 35.3, 32.9, 32.2, 31.9, 31.8, 28.3, 26.6, 26.4; IR (KBr): 3448, 3325, 2970, 1710, 1635, 1538, 1500, 1365, 1326, 1230, 1161, 1059, 850 cm^{-1}; LCMS (positive): 336.2 $(M\text{-}tBoc+H)^+$.

***N*-[1-((S)-2-Amino-3,3-dimethyl-butyryl)-piperidin-4-yl]-4-fluoro-benzamide HCl (9).** ^{1}H NMR (400 MHz, CD_3OD): δ 7.65-7.62 (m, J = 8.8 Hz, 2H), 7.12-7.07 (m, J = 8.8 Hz, 2H), 4.39-4.25 (m, 2H), 4.15-3.95 (m, 2H), 3.31-3.21 (m, 1H), 2.86-2.82 (m, 1H), 1.99-1.92 (m, 2H), 1.58-1.41 (m, 2H), 1.01-0.97 (d, 9H); ^{13}C NMR (100.6 MHz, CD_3OD): δ 168.5, 167.6, 164.9, 132.0, 131.1, 131.0, 116.4, 116.2, 58.0, 48.9, 48.2, 47.4, 46.4, 42.8, 42.5, 35.4, 34.8, 33.3, 32.2, 32.0, 26.8, 26.7; IR (KBr): 3451, 3048, 2952, 1637, 1600, 1540, 1499, 1367, 1331, 1236, 1158, 848 cm^{-1}; LCMS (positive): 336.4 $(M\text{-}HCl+H)^+$.

***N*-[1-((S)-2-{(R)-2-[(Benzyloxy-formyl-amino)-methyl]-4-methyl-pentanoylamino}-3,3-dimethyl-butyryl)-piperidin-4-yl]-4-fluoro-benzamide (16i).** To a solution of (R)-2-[(Benzyloxy-formyl-amino)-methyl]-4-methyl-pentanoic acid (0.7 g, 2.5 mmol) in DMF (7 mL) at 0 °C was added successively HOBT (0.4 g, 3.0 mmol) and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (0.57 g, 3.0 mmol). After 15 min, *N*-[1-((S)-2-Amino-3,3-dimethyl-butyryl)-piperidin-4-yl]-4-fluoro-benzamide HCl (1.67g, 4.5 mmol) and NMM (0.66 mL, 6 mmol) were added. The mixture was stirred at room temperature overnight. The mixture was poured into cold water and extracted with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. The solvents were evaporated *in vacuo*, and the residue was purified by column chromatography to yield as a white solid (78% yield). ^{1}H NMR ($CDCl_3$) δ 8.11 (bs, 0.3H, CHO-rotamer), 7.85 (bs, 0.7H, CHO-rotamer), 7.79-7.74 (m, 2H), 7.41-7.37 (m, 5H), 7.13-7.06 (m, 2H), 6.33-6.28 (m, 1H), 5.01-4.78 (m, 3H), 4.67-4.49 (m, 1H), 4.29-4.11 (m, 2H), 3.89-3.47 (m, 1H), 3.31-3.13 (m, 1H), 2.89-2.62 (m, 2H), 2.19-2.02 (m, 2H), 1.59-1.18 (m, 5H), 0.98-0.85 (m, 15H); IR (KBr): 3320, 3065, 3025, 2950, 2860, 1640, 1537, 1499, 1450, 1363, 1325, 1230, 1158, 847 cm^{-1}; LCMS (positive): 597.5 $(M+H)^+$.

4-Fluoro-*N*-[1-((S)-2-{(R)-2-[(formyl-hydroxy-amino)-methyl]-4-methyl-pentanoyl-amino}-3,3-dimethyl-butyryl)-piperidin-4-yl]-benzamide (17i). To a solution of **16i** (0.54 g, 0.9 mmol) in EtOH (50 mL) was suspended 10% Pd/C (0.2 g). The mixture was degassed under vacuum and stirred under H_2 until all the starting material was consumed. Filtration of the reaction through Celite and concentration under vacuum gave compound **17i** as a white foam (62% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.28 (bs, 0.3H, CHO-rotamer), 7.91-7.86 (m, J = 9.6 and 8.4 Hz, 2H), 7.84 (bs, 0.7H, CHO-rotamer), 7.21-7.17 (m, J = 8.4 Hz, 2H), 4.99-4.94 (m, 1H), 4.64-4.45 (m, 1H), 4.35-4.21 (m, 1H), 4.20-4.11 (m, 1H), 3.84-3.74 (m, 1H), 3.62-3.41 (m, 1H), 3.38-3.27 (m, 1H), 3.19-2.78 (m, 2H), 2.12-1.98 (m, 2H), 1.71-1.21 (m, 5H), 1.07-1.01 (m, 9H), 0.99-0.89 (m, 6H); IR (KBr): 3308, 2951, 2854, 1638, 1538, 1500, 1452, 1365, 1327, 1227, 1159, 850 cm^{-1}; LCMS (positive): 507.6 $(M+H)^+$.

Enzymatic activity assay. The enzymatic activity of PDF was evaluated by using a formate dehydrogenase (FDH)-coupled assay.[9] In this method, the formate generated by PDF from its substrate *N*-formyl-Met-Ala-Ser (formyl-MAS) is oxidized by the enzyme FDH, reducing NAD^+ to NADH which causes specific absorption at 340 nm.

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6. From GC analysis of **11** the anti/syn ratio and the de% value were obtained (Thermo Finnigan Focus GC, capillary column: ZB-5, crosslinked 5% Phenyl polysiloxane, 30 m × 0.32 mm × 0.25 μm; 80 °C, 5 min, then 10 °C/min till 280 °C; de% > 98% (P1' = cyclopentylmethyl), de% > 98% (P1' = *n*-butyl), de% > 99% (P1' = isobutyl)).
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