Tetrahedron 64 (2008) 7718–7723

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

The conformational features of palytoxin in aqueous solution

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ARTICLE INFO

Article history:
Received 23 April 2008
Received in revised form 5 June 2008
Accepted 6 June 2008
Available online 10 June 2008

Keywords:
Palytoxin
Dimer
Na/K ATPase
^{1}H NMR
NOESY

ABSTRACT

Conformational features of palytoxin and acetylated palytoxin were investigated by detailed analyses of NOESY spectra. The conformational differences between palytoxin and acetylated palytoxin may account for the difference in the assembly state of palytoxin, which exists as an associated dimer, and the acetylated derivative, which exists as a monomer in aqueous solution. Two palytoxin units in the dimer may come in contact with each other at the hydrophobic region (C21–40) and the region around two conjugated double bonds (C60–84). The amino group of palytoxin is important for biological activities via Na/K ATPase, but it was not found to be involved in the contact faces of the two palytoxin units. This information should aid in revealing how palytoxin interacts with Na/K ATPase.

1. Introduction

Marine organisms often produce unique secondary metabolites that have not been observed in terrestrial organisms. In particular, large polyol and polyether compounds are architecturally intriguing, not just because of their size. These compounds are composed of a long carbon backbone with poly-oxygen functionalities, so called super-carbon-chain compounds.[1]

Coelenterate toxin palytoxin (PTX, Fig. 1),[2] which is a representative super-carbon-chain compound, shows potent toxicity with an LD_{50} value of 450 ng/kg against mice. It has been suggested that PTX interacts with Na/K ATPase[3] and changes the Na/K pump to the pore. PTX is therefore a very useful tool for functional studies of Na/K ATPase.[4]

Knowledge of the entire shape of PTX would likely provide important information regarding its mechanism of interaction with Na/K ATPase. For this purpose, we performed small-angle X-ray scattering (SAXS) experiments of PTX in aqueous solution with an intense synchrotron X-ray source.[5] From the SAXS results, PTX was shown to exist as a dimer whereas an acetylated PTX derivative (NAcPTX), whose biological activity via Na/K ATPase was over 100 times weaker,[6] existed as a monomer in aqueous solution. The outline of the molecular shape was also revealed by model simulations, but it was unclear which part of the two PTX molecules interacted with each other. The relationship between the dimerization and the biological activity was interesting. We describe here the results of our analyses of the conformation of PTX and NAcPTX by NMR experiments.

2. Results and discussion

2.1. The chemical shift assignment of PTX and NAcPTX

We commenced this study with assignment of ^{1}H NMR of PTX and NAcPTX in 1.5 mg/mL of deuterium oxide. PTX exists as a dimer and NAcPTX exists as a monomer at this concentration.[5] The chemical shift assignment of PTX and NAcPTX has been reported only with aqueous methanol solution.[7] The use of deuterium oxide as a solvent reduced the sharpness of the PTX signals, but the use of deuterium oxide enabled a direct comparison of conformational information from ^{1}H NMR and that obtained from SAXS experiments in aqueous solution. The ^{1}H NMR signals of PTX and NAcPTX were assigned by DQF-COSY spectra.

The low concentrations of these samples had prevented such an assignment by use of HOHAHA spectra.

The results of the chemical shift assignment for PTX and NAcPTX are shown in Table 1. Protons at a hydrophobic region (H34–39) of PTX and NAcPTX were not assignable because these signals were

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doi:10.1016/j.tet.2008.06.025

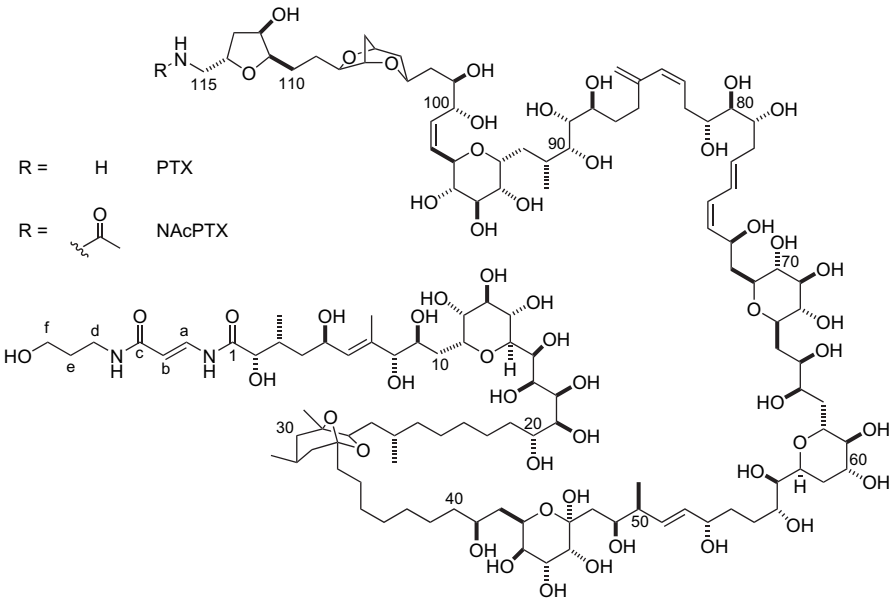

Figure 1. The chemical structures of PTX and NAcPTX.

heavily overlapped. However, protons in the other regions of PTX and NAcPTX could be assigned to chemical structures. The ^{13}C NMR spectra of PTX and NAcPTX were not available because of their low concentrations.

2.2. NOESY spectrum analyses of PTX and NAcPTX

In order to obtain further information regarding the conformation of the associated PTX dimer and NAcPTX monomer, we analyzed NOESY spectra. NOESY experiments were conducted under the same conditions for ^{1}H NMR and DQF-COSY. NOESY correlations are shown in Figure 2.

The correlations in PTX were different from those in NAcPTX in some ways. Most notably, regions of H66–70 and H92–96 proved to be very proximate in PTX. In the case of NAcPTX, correlations among these regions were not observed. Another important feature of PTX is the correlations from the nitrogen terminal furan to H50 and to H7-Me. The corresponding furan of NAcPTX, on the other hand, exhibited correlations with protons at H58 and H61. H12 and H55–56 moieties were shown to exist closely in space for both PTX and NAcPTX.

2.3. The difference in chemical shift

The conformational differences between PTX and NAcPTX were also reflected in their chemical shifts (Table 1). Protons with over 0.1 ppm difference between PTX and NAcPTX are indicated by the filled circles in Figure 3. The marked protons are in four areas: (i) H11–16, (ii) H48, (iii) H62–65, and (iv) H111–115. As described in Section 2.2, NOESY correlations were observed between the following areas: PTX (areas (i) and (ii), and areas (ii) and (iv)), NAcPTX (areas (i) and (iii) and areas (iii) and (iv)).

2.4. Chain model representation of conformations of NAcPTX

Kishi has proposed an entire conformation of the PTX derivative using a chain model.[8] They conducted NMR analysis of synthetic small segments for PTX and put these results together to estimate the entire conformation of PTX. Consequently, the result was a rough estimation and there may be a room for improvement. For instance, the effects of acylation at the nitrogen terminal were not fully discussed in the report.

We are proposing a plausible conformation of NAcPTX as depicted in Figure 4. The model was built to satisfy all the proximity data obtained from NOESY experiments. Because little evidence was collected from the NOESY experiments regarding the terminal regions of the horseshoe-like shaped molecule, we have adopted here the proposal by Kishi that the chain would turn at each ether ring and at the conjugated double bonds.[8] In this way, the chain model of NAcPTX was drawn to fit well with the SAXS model that had been proven experimentally.[5] The chain model is also in accordance with the SAXS model in that half of the mass is concentrated at one edge of this horseshoe-like molecule. A distinct difference between models by Kishi and us is that the amino terminal (C115) is located near two ether rings (C11–15 and C58–62), whereas they are far from these rings in Kishi's model. Considering the size of the SAXS model ($30.6\times23.4\times13.0$ Å),[5] the distance between two terminals of the carbon chain should be different from that estimated previously (ca. 30 Å).[8]

2.5. Chain model representation of conformations of associated PTX dimer

In the case of PTX, NOESY correlations of PTX indicated that the nitrogen terminal (C115) was located far from two ether rings (C11–15 and C58–62), because three NOESY correlations were observed, between H7–Me and H113, between H12 and H55, and between H50 and H113. Two ether rings (C66–70 and C92–96) were proven to be located near each other. A plausible chain model of PTX is shown in Figure 5. Although the rough shapes of the monomer unit of PTX and NAcPTX look similar, the global conformations of these molecules are actually quite different.

In the model from the SAXS experiments, two PTX molecules were attached at both terminals of the horseshoe-like shape. The hydrophobic region (H21–40) and the region around two conjugated double bonds (H60–84) correspond to two terminals in our model. Two terminals of an SAXS model of NAcPTX could be distinguished from each other due to the mass differences, but the resolution of the SAXS model for PTX

Table 1
Chemical shift data of PTX and NAcPTX

Position	PTX	NAcPTX	Chemical shift difference[a]	Position	PTX	NAcPTX	Chemical shift difference
	^{1}H (δ)	^{1}H (δ)			^{1}H (δ)	^{1}H (δ)	
1	—	—	—	40	1.44	1.41	−0.03
2	3.96	4.02	0.06	41	3.77	3.71	−0.06
3	1.99	2.02	0.03	42	1.71, 1.35	1.72, 1.32	0.01, −0.03
3-Me	0.73	0.75	0.02	43	4.24	4.24	0.00
4	1.60, 1.31	1.69, 1.30	0.09, −0.01	44	3.66	3.67	0.01
5	4.41	4.45	0.04	45	3.98	3.91	−0.07
6	5.32	5.38	0.06	46	3.66	3.69	0.03
7	—	—	—	47	—	—	—
7-Me	1.54	1.61	0.07	48	1.68	1.79	0.11
8	3.81	3.83	0.02	49	3.78	3.84	0.06
9	3.86	3.75	0.09	50	2.18	2.21	0.03
10	1.96, 1.59	1.97, 1.61	0.01, 0.02	50-Me	0.87	0.89	0.02
11	4.06	4.19	0.13	51	5.44	5.45	0.01
12	3.67	3.55	−0.12	52	5.38	5.38	0.00
13	3.60	3.49	−0.11	53	3.92	3.99	0.07
14	3.60	3.58	−0.02	54	1.66	1.68	0.02
15	3.65	3.55	−0.10	55	1.62, 1.36	1.57, 1.35	−0.01, −0.05
16	3.77	3.90	0.13	56	3.71	3.77	0.06
17	3.87	3.90	0.03	57	3.78	3.82	0.04
18	3.53	3.61	0.08	58	3.78	3.81	0.03
19	3.67	3.67	0.00	59	2.18, 1.58	2.15, 1.59	0.01, −0.03
20	3.75	3.70	−0.05	60	3.75	3.78	0.03
21	1.36	1.39, 1.32	0.03, −0.04	61	3.04	3.05	0.01
22	1.36	1.41, 1.28	0.05, −0.08	62	3.68	3.79	0.11
23	1.58	1.49	−0.09	63	1.93, 1.63	1.99, 1.61	0.06, −0.02
24	1.32	1.26	−0.06	64	3.58	3.68	0.10
25	1.23	1.22	−0.01	65	3.57	3.70	0.13
26	1.42	1.47	0.05	66	1.95, 1.39	1.98, 1.36	0.03, −0.03
26-Me	0.73	0.77	0.04	67	3.32	3.35	0.03
27	1.29, 0.81	1.34, 0.78	0.05, −0.03	68	3.05	3.09	0.04
28	3.92	3.93	0.01	69	3.31	3.37	0.06
29	—	—	—	70	3.04	3.08	0.04
29-Me	1.06	1.09	0.03	71	3.47	3.51	0.04
30	1.04	1.08	0.04	72	1.95, 1.39	1.98, 1.40	0.03, 0.01
31	1.85	1.89	0.04	73	4.68	4.72	0.04
31-Me	0.76	0.79	0.03	74	5.27	5.29	0.02
32	1.55, 0.98	1.59, 1.00	0.04, 0.02	75	5.98	5.98	0.00
33	—	—	—	76	6.32	6.32	0.00
34	[b]	[b]	—	77	5.68	5.68	0.00
35	[b]	[b]	—	78	2.24	2.26	0.02
36	[b]	[b]	—	79	3.75	3.79	0.04
37	[b]	[b]	—	80	3.24	3.27	0.03
38	[b]	[b]	—	81	3.61	3.65	0.04
39	[b]	[b]	—	82	2.48, 2.32	2.52, 2.34	0.04, 0.02
83	5.52	5.56	0.04	103	4.04	4.06	0.02
84	5.88	5.91	0.03	104	1.59, 1.30	1.65, 1.32	0.06, 0.02
85	—	—	—	105	4.46	4.49	0.03
85′	4.98, 4.82	5.01, 4.87	0.03, 0.05	106	1.75, 1.62	1.79, 1.65	0.04, 0.03
86	2.14	2.15	0.01	107	4.16	4.18	0.02
87	1.57, 1.44	1.58, 1.49	0.01, 0.05	108	4.23	4.27	0.04
88	3.61	3.61	0.00	109	1.68	1.70	0.02
89	3.42	3.45	0.03	110	1.48	1.47	−0.01
90	3.29	3.33	0.04	111	3.61	3.76	0.15
91	1.57	1.59	0.02	112	4.05	4.18	0.13
91-Me	0.75	0.78	0.03	113	2.03, 1.79	1.95, 1.72	−0.08, −0.07
92	1.97, 1.34	2.02, 1.30	0.05, −0.04	114	4.26	4.22	−0.04
93	4.00	4.03	0.03	115	2.91, 2.82	3.19, 3.16	0.28, 0.34
94	3.61	3.64	0.03	a	7.56	7.56	0.00
95	3.52	3.56	0.04	b	5.80	5.81	0.01
96	3.14	3.16	0.02	c	—	—	—
97	4.13	4.16	0.03	d	3.17	3.21	0.04
98	5.46	5.49	0.03	e	1.62	1.65	0.03
99	5.61	5.65	0.04	f	3.48	3.51	0.03
100	4.18	4.20	0.02	C═O (Ac)	—	—	—
101	3.47	3.53	0.06	Me (Ac)	—	1.86	—
102	1.45	1.44	−0.01				

[a] Subtraction of chemical shifts of PTX from those of NAcPTX in parts per million.
[b] Not assigned.

was not sufficient to identify the association mode (Fig. 5a or 5b). Some protons in a possible contact face region (H34–39) could not be assigned because of overlap with the ^{1}H NMR signals; therefore, NOESY correlations of the protons in this area were not assignable. So far no obvious intermolecular NOESY correlations between assigned protons in the hydrophobic region and the protons around two conjugated double bonds have been observed.

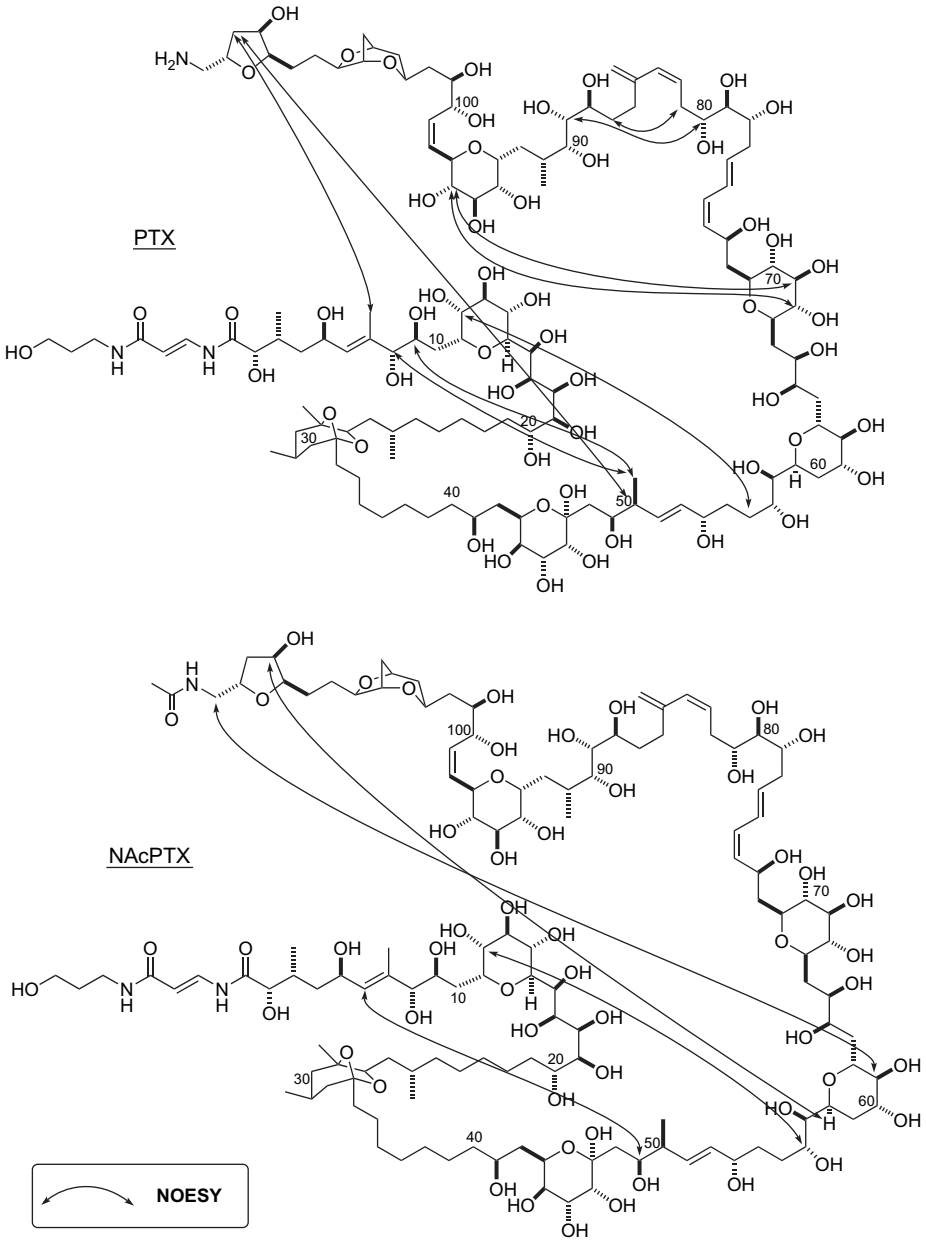

Figure 2. Selected NOESY correlations of PTX and NAcPTX. Correlations between adjacent protons are omitted.

When we began this NMR-based study, the terminal amino group was anticipated to be involved in a contact face of PTX dimer because its acetylation inhibits the dimerization. In both of our chain models (Figs. 4 and 5a or 5b), the nitrogen terminal is not located at the edge of horseshoe-like molecule. These results would suggest that the acetylation of the amino group causes a change in the global conformation that influences the associative nature of PTX. We are currently investigating the effects of the acetylation of the amino group with basic amino acids such as lysine or arginine to prove the importance of positive charge at the nitrogen terminal of PTX.

3. Conclusion

We have described the conformational features of PTX and NAcPTX from extensive NMR experiments, which were not available from previous SAXS experiments. Further efforts toward establishing a detailed conformation of PTX are in progress.

4. Experimental

4.1. General

NMR spectra were recorded on a JEOL JNM-A600 spectrometer (600 MHz for proton). Chemical shifts are reported in parts per million (ppm) in hertz relative to the solvent peaks, δ_H 4.67 (HDO). The number of data points in DQF-COSY and NOESY spectra was 1024×512, and this was finally zero-filled to 2048×1024.

4.2. Sample preparation

PTX was isolated from *Palythoa tuberculosa*.[9] NAcPTX was prepared from PTX by acetylation with *p*-nitrophenyl acetate.[9] PTX and

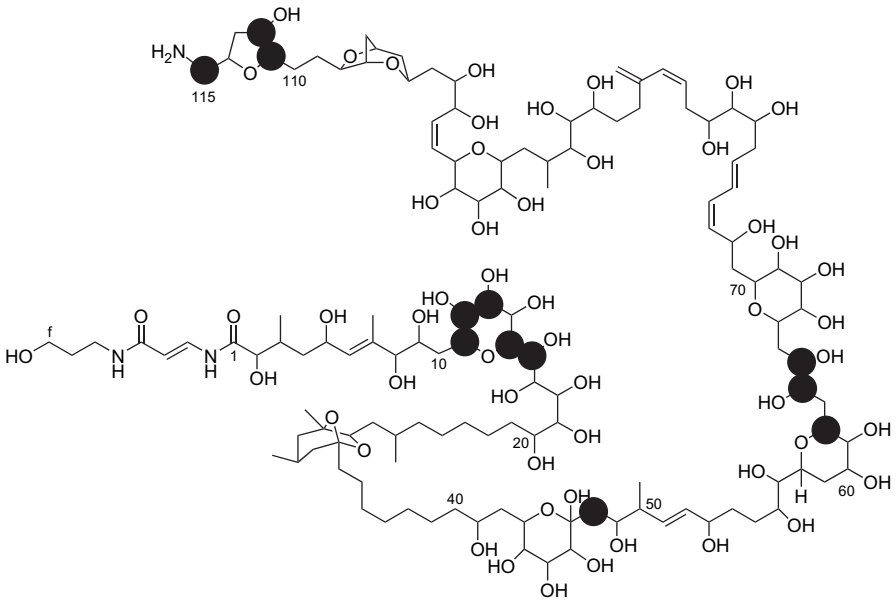

Figure 3. The proton that had an over 0.1 ppm difference of the chemical shift between PTX and NAcPTX was shown by the filled circle.

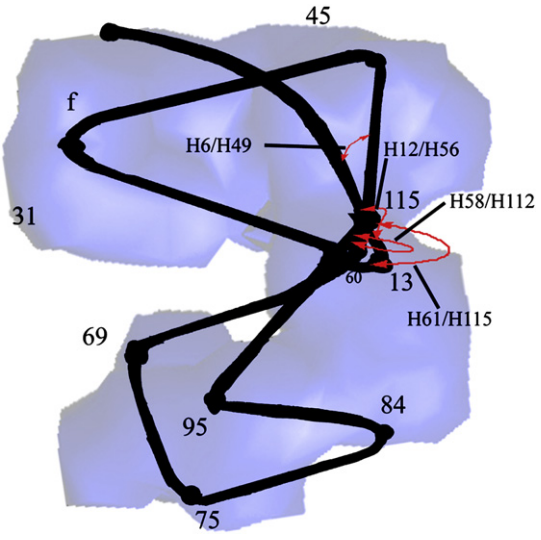

Figure 4. A schematic illustration of NAcPTX (bold line) superimposed on its conformation model in solution obtained from an SAXS experiment.[5] The size of the SAXS model is 30.6×23.4×13.0 Å.[5] Selected NOESY correlations that prove the proximity of carbon chains are shown by two-headed arrows.

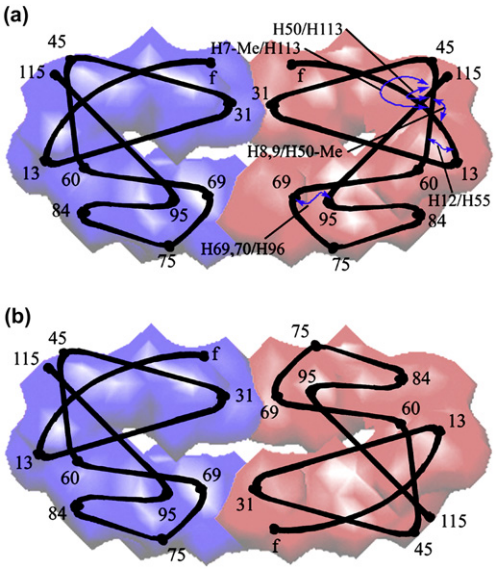

Figure 5. Plausible schematic illustrations of PTX (bold line) superimposed on its conformation model in solution obtained from an SAXS experiment.[5] The size of the SAXS model is 52.3×22.0×15.1 Å.[5] Selected NOESY correlations that prove the proximity of carbon chains are shown by the two-headed arrows.

NAcPTX were dissolved in deuterium oxide. The measured concentrations of both PTX and NacPTX were 1.5 mg/mL.

Acknowledgements

We thank Prof. Tetsuro Fujisawa (Gifu University) for advising about the study of the molecular shape of PTX. This study was supported by a Grant-in-Aid for Scientific Research (16GS0206 and 16310150) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Supplementary data

DQF-COSY and NOESY spectra of PTX and NAcPTX. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.06.025.

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