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Design, synthesis, and biological evaluation of novel analogues of archazolid: A highly potent simplified V-ATPase inhibitor

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Abstract—Novel analogues of the V-ATPase inhibitors archazolid A and B with modifications of the free hydroxyl groups and the side chain were designed by molecular modeling, synthesized by derivatization of the parent natural product and evaluated for V-ATPase inhibition and growth inhibition of murine connective tissue cells.

The myxobacterium *Archangium gephyra* is the natural source of the archazolids A and B (**1**, **2**, Fig. 1), poly-unsaturated 24-membered macrolides of polyketide origin.[1–3] They are highly potent cytostatic agents, which inhibit proliferation of a range of cell lines at nanomolar concentrations. This effect is caused by selective inhibition of vacuolar-type ATPases (V-ATPases).[4–6] These proton-translocating enzymes present an important target from the perspective of medicinal chemistry, as their functionality is closely associated with various diseases such as cancer[7] or renal acidosis,[8] which renders the development and molecular understanding of selective and synthetically accessible V-ATPase inhibitors an important research goal. Recently, the relative and absolute configuration of the archazolids has been determined by extensive high-field NMR-studies and chemical derivatization.[9] For further advancing the archazolids, structure–activity data are vital. However, so far, no such data have been reported.

Herein we describe the synthesis of a first set of carefully selected archazolid analogues with modifications of the hydroxyl groups at C-7, C-15, and C-1′. We disclose the cell growth inhibitory activity and V-ATPase inhibition of all of these compounds relative to other V-ATPase inhibitors (apicularen, bafilomycin) and report a highly potent, structurally simplified analogue.

Our starting point for analogue studies were conformational analyses (MMFS, MacroModel 8.5) of archazolid and related structures, which were carried out both in vacuo and in solution (water).[10] These calculations revealed that functionalization of the free 7-OH group only has a minor impact on the respective 3D-structures. Likewise, changes in the side chain, for example by removing the carbamate, only had a minor influence on the macrocyclic conformation, as expected. These findings prompted us to evaluate the general importance of the 7-OH and the side-chain carbamate on biological activity, together with preparing and evaluating these analogues with derivatives at C-15.

As a first target, we decided to selectively modify one of the free macrocyclic hydroxyls of archazolid A (Scheme 1). It was found that the 7-OH was more reactive to electrophiles than the hydroxyl at C-15, which may be due to a certain degree of hydrogen bonding of 15-OH to 17-OMe. The space-filling derivative **3** and the *para*-nitrobenzoate derivative **4** were chosen as sterically and electronically related mimics of previously reported concanolide analogues.[11] After optimizing conditions, silylation (with *tert*-butyldimethylsilyl chloride, TBSCl) and benzoylation of **1** (by treatment with *p*-nitrobenzoyl chloride, PNBzCl, and 4-dimethylaminopyridine, DMAP, in dichloromethane) proceeded smoothly and gave derivatives **3** and **4** in good yields.[12]

Keywords: Archazolid; Analogues; V-ATPase; Structure–activity data; Modeling.
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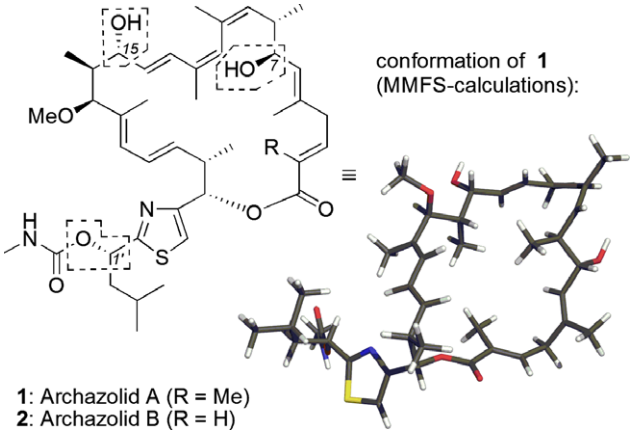
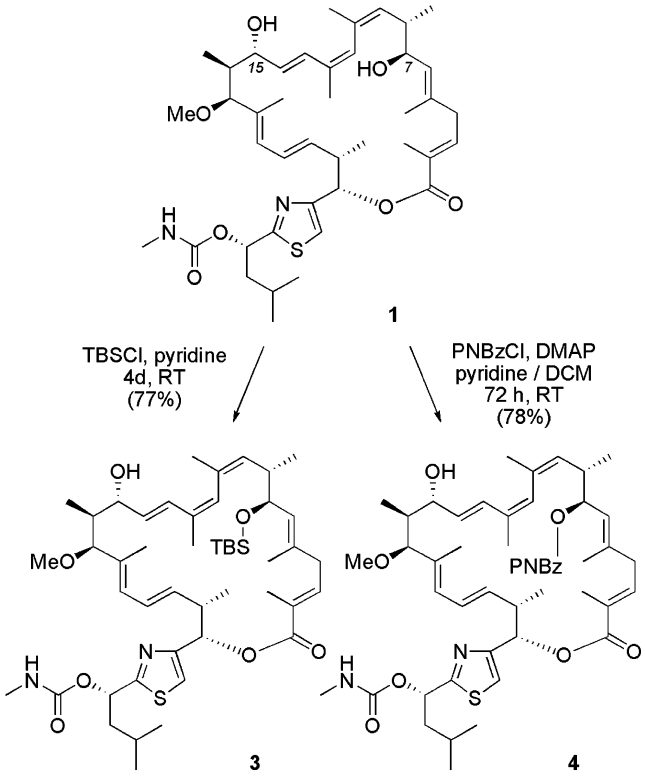
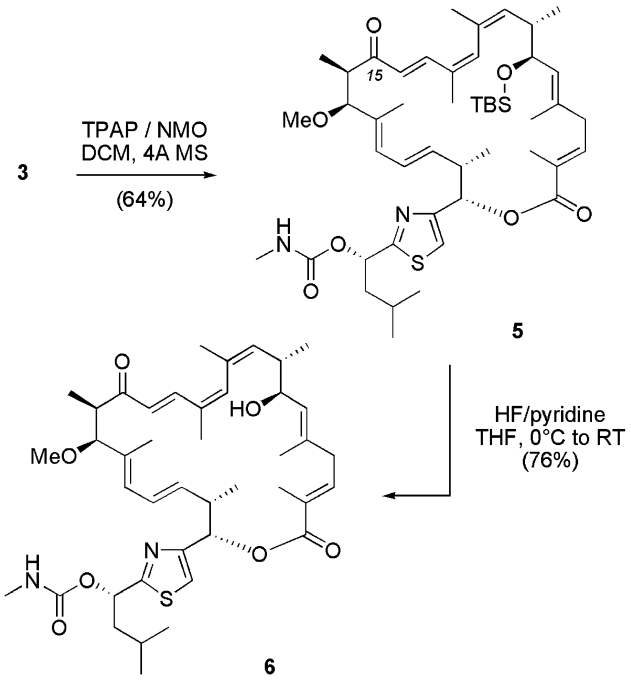


Figure 1. Archazolids A and B: potent V-ATPase inhibitors.



Scheme 1. Synthesis of 7-*O*-analogues **3** and **4**.

Synthetic access to archazolid derivative **3** opened the possibility of elaborating C-15 analogues. A certain degree of conformational flexibility in solution around the C15–C16)-bond has recently been disclosed for the archazolids by NMR studies.[9,13] Therefore, structural modifications in the 'north-western' region are expected to have an impact on the overall 3D-structure and flexibility of these anti-proliferative agents and might allow a conformation-based modulation of biological function. Along these lines, switching the saturated C-15 carbon to an sp^2-center was expected to have a significant conformational influence. Thus, the corresponding 15-keto derivative (dehydroarchazolid **6**, see Scheme 2) was chosen as a rewarding analogue. The synthesis of **6**



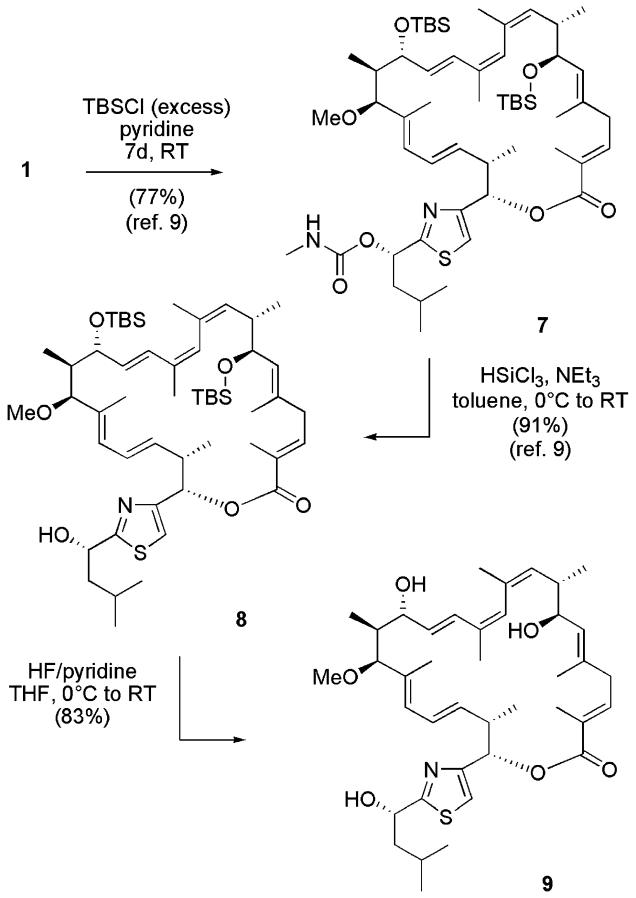
Scheme 2. Synthesis of dehydroarchazolid **6**.

proceeded smoothly by oxidation of **3** with tetrapropylammonium perruthenate (TPAP)/*N*-Methylmorpholin-*N*-oxid (NMO) and subsequent cleavage of the 7-*O*-TBS group by the aid of HF/pyridine.

A key step in our recently disclosed stereochemical assignment of the archazolids involved a selective cleavage of the side-chain carbamate of bis-TBS-derivative **7**,[9] by a method of Pirkle and Hauske using trichlorosilane in the presence of triethylamine,[14] to give compound **8** (Scheme 3). Within these studies, this now enabled the preparation of the truncated archazolid analogue **9** by deprotection of **8** using again HF/pyridine. Overall, this simplified derivative is available from archazolid A in a very efficient manner.

For biological evaluation of the foregoing analogues, we first checked the inhibitory efficacy on purified V-ATPase holoenzyme, from the midgut of the tobacco hornworm (Table 1). For this purpose, V-ATPase was purified according to published procedures[11] and a standard V-ATPase assay was employed with or without inhibitors at various concentrations.[5] Inorganic phosphate, produced in the assays of V-ATPase, was measured according to the protocol of Wieczorek et al.[15] Under these conditions, the truncated analogue **9** and the keto-derivative **6** were shown to be potent V-ATPase inhibitors with activities, that are only 8 and 20 times lower than those for the archazolids. 7-*O*-derivatives (**3**, **4**), on the contrary, are much less effective and the inhibitory effect is decreased by three orders of magnitude.

Table 1 also summarizes the inhibitory effect of archazolid and its analogues, as compared with apicularen A and bafilomycin A, on the growth of the mammalian



Scheme 3. Synthesis of the truncated archazolide analogue **9**.

Table 1. Effect on in vitro V_1/V_o holoenzyme inhibition and inhibitory effects of ligands on growth of mammalian murine connective tissue cell line

Compound	Enzyme inhibition V_1/V_o holoenzyme: IC_{50},[a] nmol/mg enzyme	Growth inhibition L-929 IC_{50},[d] nM
Archazolid A (**1**)	0.6[b]	0.81
Archazolid B (**2**)	0.6[c]	1.1
3	140	940
4	250	290
6	13	>5000
9	5	14
Apicularen A	0.8[c]	4.5[c]
Bafilomycin A	0.5[e]	3.2[c]

[a] The specific enzyme activity of the controls without inhibitors was 1.5 ± 0.2 μmol $mg^{-1}min^{-1}$.

[b] 0.8 nmol/mg enzyme accords to 10 nM.

[c] Value taken from Ref. 5.

[d] L-929 mouse cell line DSM ACC 2 was from the German collection of Microorganisms and Cell Cultures (DSMZ). Growth inhibition was measured as previously described and metabolic activity was determined after 5 days using the MTT assay.[16]

[e] Value taken from Ref. 11.

tissue cell line L-929. Among the derivatives tested, the most potent one is again the simplified structure **9**, which indicates that the side-chain carbamate is not important for biological activity, while the inhibitory effect of **3** and **4** is again dramatically reduced, suggesting a free 7-OH to be part of the pharmacophore. While these data are in agreement with the in vitro inhibitory effects on the holoenzmye, analogue **6** was essentially inactive in this assay having an IC_{50} above 5 μM. This highly interesting and reverse behavior of **6** could possibly be associated with a modified membrane permeability as compared to all other analogues. Significantly, the truncated analogue **9** is only 17-fold less potent in this assay as compared to **1** and of similar potency to apicularen A[5] and bafilomycin A.[5,11] Notably, it represents the most active natural product derived V-ATPase inhibitor known so far.

In conclusion, based on molecular modeling, we have developed a first series of analogues of the archazolids with modifications in the macrocycle and the side chain. The synthesis of these compounds was enabled by selective derivatizations of archazolid A. This has led to the discovery of a highly potent and structurally simplified V-ATPase inhibitor, 1′-descarbamoyl-archazolid A (**9**), which presents the most potent synthetic V-ATPase inhibitor known so far. Furthermore, it contains an additional free hydroxyl, which may be used to further modulate its biological activity. Functionalization of the hydroxyl at C-7 leads to significant loss of activity, which suggests this area to be part of the pharmacophoric region. One of the analogues studied, 15-dehydroarchazolid (**6**), has high in vitro, but essentially no in vivo, activity. Possibly, this effect could be related to a modified 3D conformation of **6**. The 20-fold decrease in sensitivity of the V-ATPase to dehydroarchazolid **6** as compared to archazolid A (**1**) indicates that the hydroxyl group at C-15 may have an important function for binding of the inhibitor to the protein. In total, these results warrant further research of the anti-V-ATPase potential and solution structure of these antimitotic agents a rewarding task.

Acknowledgments

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12. All new compounds had spectroscopic data in full support of the assigned structures. ^{1}H NMR spectroscopic data: Compound **3**: ^{1}H NMR δ (600 MHz, CD_3OD): 0.03 (s, 3 H, Si–CH_3), 0.06 (s, 3 H, Si–CH_3), 0.78 (d, J = 7.3 Hz, 3 H, 16-CH_3), 0.88 (d, J = 6.8 Hz, 3 H, 8-CH_3), 0.91 (s, 9 H, Si–$C(CH)_3$), 1.01 (d, J = 6.80 Hz, 3 H, 8′-H_3), 1.03 (d, J = 6.4 Hz, 3 H, 7′-H_3), 1.12 (d, J = 6.8 Hz, 3 H, 22-CH_3), 1.61 (d, J = 1.1 Hz, 3 H, 18-CH_3) 1.72 (d, J = 1.1 Hz, 3 H, 5-CH_3), 1.75 (ddq, J = 8.7, 4.2, 6.8 Hz, 1 H, 16-H), 1.78 (s, 3 H, 10-CH_3), 1.80 (m, 2 H, 5′-H, 6′-H), 1.84 (dd, J = 8.3, 4.9 Hz, 1 H, 5′-H), 1.91 (s, 3 H, 2-CH_3), 1.93 (d, J = 1.1 Hz, 3 H, 12-CH_3), 2.30 (ddq, J = 9.8, 8.6, 6.8 Hz, 1 H, 7-H), 2.74 (s, 3 H, 2″-H_3), 2.95 (dd, J = 15.1, 6.8 Hz, 1 H, 4-H), 3.00 (dd, J = 14.4, 9.1 Hz, 1 H, 4-H), 3.06 (ddq, J = 7.6, 4.5, 6.8 Hz, 1 H, 22-H), 3.13 (s, 3 H, 17-OCH_3), 3.29 (d, J = 8.7 Hz, 1 H, 17-H), 4.08 (dd, J = 8.9, 8.9 Hz, 1 H, 7-H), 4.20 (dd, J = 7.2, 4.2 Hz, 1 H, 15-H), 5.16 (dt, J = 10.1, 1.2 Hz, 1 H, 9-H), 5.19 (d, J = 9.1 Hz, 1 H, 6-H), 5.55 (dd, J = 15.1, 7.6 Hz, 1 H, 21-H), 5.80 (dd, J = 15.9, 7.9 Hz, 1 H, 14-H), 5.80 (s, 1 H, 11-H), 5.83 (d, J = 9.4 Hz, 1 H, 19-H), 5.96 (d, J = 4.5 Hz, 1 H, 23-H), 6.03 (dd, J = 9.1, 4.9 Hz, 1 H, 1′-H), 6.06 (dd, J = 14.7, 10.6 Hz, 1 H, 20-H), 6.38 (d, J = 15.9 Hz, 1 H, 13-H), 6.87 (ddd, J = 8.3, 6.8, 1.5 Hz, 1 H, 3-H), 7.20 (s, 1 H, 4′-H) ppm. - Compound **4**: ^{1}H NMR δ (600 MHz, CD_3OD): 0.79 (d, J = 7.0 Hz, 3 H, 16-CH_3), 1.04 (m, J = 6.8 Hz, 3 H, 8-CH_3), 0.98 (d, J = 6.8 Hz, 7′-H_3), 1.00 (d, J = 6.8 Hz, 8′-H_3), 1.11 (d, J = 6.8 Hz, 3 H, 22-CH_3), 1.64 (s, 3 H, 18-CH_3), 1.65 (s, 3 H, 10-CH_3),1.72 (ddq, J = 8.5, 3.2, 7.0 Hz, 16-H), 1.77 (m, 6′-H), 1.82 (m, 5′-H), 1.86 (m, 5′-H), 1.89 (d, J = 0.8 Hz, 3 H, 5-CH_3), 1.91 (s, 3 H, 2-CH_3), 1.99 (d, J = 0.9 Hz, 3 H, 12-CH_3), 2.69 (ddq, J = 9.7, 9.6, 6.8 Hz, 8-H), 2.75 (s, 2″-H_3), 2.88 (dd, J = 14.3, 7.2 Hz, 4-H), 3.04 (ddq, J = 7.4, 5.3, 6.8 Hz, 22-H), 3.06 (dd, J = 14.3, 7.9 Hz, 4-H), 3.17 (s, 3-H, 17-OCH_3), 3.31 (d, J = 8.5 Hz, 1 H, 17-H), 4.30 (dd, J = 8.2, 3.2 Hz, 1 H, 15-H), 5.22 (d, J = 9.6 Hz, 1 H, 9-H), 5.29 (d, J = 10.0 Hz, 1 H, 6-H), 5.51 (dd, J = 15.4, 7.3 Hz, 1 H, 21-H), 5.53 (dd, J = 9.7, 9.7 Hz, 1 H, 7-H), 5.69 (s, 1 H, 11-H), 5.80 (d, J = 11.1 Hz, 1 H, 19-H), 5.88 (dd, J = 16.0, 7.5 Hz, 1 H, 14-H), 5.96 (d, J = 5.3 Hz, 1 H, 23-H), 6.03 (dd, J = 8.7, 4.5 Hz, 1 H, 1′-H), 6.13 (dd, J = 15.4, 10.8 Hz, 1 H, 20-H), 6.45 (d, J = 15.6 Hz, 1 H, 13-H), 6.81 (dd, J = 8.1, 7.2 Hz, 1 H, 3-H), 7.20 (s, 1 H, 4′-H), 8.26 (d, J = 9.0 Hz, 2 H, PNB-H_2), 8.40 (d, J = 9.0 Hz, 2 H, PNB-H_2) ppm. Compound **6**: ^{1}H NMR δ (600 MHz, CD_3OD): 0.94 (d, J = 7.2 Hz, 3 H, 8-CH_3), 0.96 (d, J = 6.8 Hz, 3 H, 16-CH_3), 1.00 (d, J = 6.8 Hz, 3 H, 8′-H_3), 1.01 (d, J = 6.4 Hz, 3 H, 7′-H_3), 1.12 (d, J = 7.2 Hz, 3 H, 22-CH_3), 1.72 (s, 3 H, 18-CH_3), 1.77 (d, J = 1.1 Hz, 3 H, 5-CH_3), 1.81 (m, 1 H, 6′-H), 1.84 (s, 3 H, 10-CH_3), 1.91 (s, 3 H, 2-CH_3), 1.94 (m, 2 H, 5′-H), 1.96 (d, J = 1.1 Hz, 3 H, 12-CH_3), 2.48 (ddq, J = 9.8, 9.5, 7.0 Hz, 1 H, 8-H), 2.74 (s, 3 H, 2″-H_3), 2.97 (dd, J = 15.4, 7.5 Hz, 1 H, 4a–H), 3.08 (dd, J = 15.5, 7.5 Hz, 1 H, 4 b–H), 3.13 (s, 3 H, 17-OCH_3), 3.19 (d, J = 7.2 Hz, 1 H, 16-H), 3.68 (d, J = 7.2 Hz, 1 H, 17-H), 4.22 (dd, J = 9.1, 6.0 Hz, 1 H, 7-H), 5.26 (d, J = 9.8 Hz, 1 H, 6-H), 5.28 (d,J = 9.8 Hz, 1 H, 9-H), 5.64 (dd, J = 15.3, 8.5 Hz, 1 H, 22-H), 5.90 (d, J = 10.6 Hz, 1 H, 19-H), 5.95 (d, J = 5.3 Hz, 1 H, 23-H), 6.04 (dd, J = 9.3, 4.7 Hz, 1 H, 1′-H), 6.17 (dd, J = 15.3, 10.8 Hz, 1 H, 20-H), 6.23 (d, J = 15.9 Hz, 1 H, 14-H), 6.35 (s, 1 H, 11-H), 7.01 (dd, J = 7.6, 7.6 Hz, 1 H, 3-H), 7.17 (s, 1 H, 4′-H), 7.59 (d, J = 16.2 Hz, 1 H, 13-H). - Compound **9**: ^{1}H NMR δ (600 MHz, CD_3OD): 0.74 (d, J = 7.2 Hz, 3 H, 16-CH_3), 0.84 (d, J = 6.8 Hz, 3 H, 8-CH_3), 1.02 (d, J = 6.8 Hz, 3 H, 7′-CH_3), 1.03 (d, J = 6.4 Hz, 3 H, 8′-CH_3), 1.14 (d, J = 7.2 Hz, 3 H, 22-CH_3), 1.65 (s, 3 H, 18-CH_3), 1.73 (d, J = 1.1 Hz, 3 H, 5-CH_3), 1.77 (m, 1 H, 6′-H), 1.80 (ddq, J = 9.0, 3.0, 7.0 Hz, 1 H, 16-H), 1.80 (s, 3 H, 10-CH_3), 1.90 (s, 3 H, 2-CH_3), 1.93 (d, J = 1.1 Hz, 3 H, 12-CH_3), 2.06 (m, 2 H, 5′-H), 2.30 (ddq, J = 9.4, 9.1, 6.8 Hz, 1 H, 8-H), 2.91 (dd, J = 14.7, 7.6 Hz, 1 H, 4a–H), 3.03 (dd, J = 14.7, 8.3 Hz, 1 H, 4 b–H), 3.08 (ddq, J = 7.0, 4.9, 7.2 Hz, 1 H, 22-H), 3.17 (s, 3 H, 17-OCH_3), 3.42 (d, J = 8.7 Hz, 1 H, 17-H), 4.04 (dd, J = 9.1, 9.1 Hz, 1 H, 7-H), 4.31 (dd, J = 6.2, 3.0 Hz, 1 H, 15-H), 5.20 (d, J = 9.4 Hz, 1 H, 6-H), 5.28 (d, J = 9.4 Hz, 1 H, 9-H), 5.37 (m, 1 H, 1′-H), 5.68 (dd, J = 15.3, 7.0 Hz, 1 H, 21-H), 5.79 (dd, J = 16.1, 6.2 Hz, 1 H, 14-H), 5.81 (s, 1 H, 11-H), 5.87 (d, J = 11.0 Hz, 1 H, 19-H), 5.94 (d, J = 4.9 Hz, 1 H, 23-H), 6.24 (dd, J = 15.1, 10.6 Hz, 1 H, 20-H), 6.56 (d, J = 16.2 Hz, 1 H, 13-H), 6.82 (dd, J = 7.9, 7.9 Hz, 1 H, 3-H) ppm.
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