Bioorganic & Medicinal Chemistry Letters 25 (2015) 4020–4023

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of dialkyl diacetylene diols with potent cancer chemopreventive activity

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ARTICLE INFO

Article history:
Received 15 February 2015
Revised 17 April 2015
Accepted 28 May 2015
Available online 6 June 2015

Keywords:
Cancer
Chemoprevention
Diyne
Quinone reductase
Stereoisomeric

ABSTRACT

An increasing importance of chemoprevention for controlling cancer risks prompted the discovery of new active cancer chemopreventive agents. In this study, we designed and synthesized substituted hexa-2,4-diyne-1,6-diols, more structurally simplified, tunable, and easily preparable than natural gymnasterkoreaynes, and evaluated their cancer chemopreventive activities by measuring concentration of doubling quinone reductase activity (CD), cell viability, and chemopreventive index (CI). Most of the diols exhibited good CD activity and low cytotoxicity. In particular, tetradeca-5,7-diyne-4,9-diol and 2-methyltetradeca-5,7-diyne-4,9-diol showed the best cancer chemopreventive activity, approximately equipotent to that of sulforaphane. And, by synthesizing optically active stereoisomers of selected active compounds, the effect of stereochemistry was also studied. Eventually, we produced a chemopreventive compound for in vivo study.

Cancer chemoprevention is defined as the inhibition, retardation, and/or reversal of the carcinogenesis steps that include initiation, promotion, and progression by chemicals with otherwise low cytotoxicity.[1–3] Chemoprevention was categorized in three main areas by Russo group: (1) prevention of carcinogenesis in healthy individuals; (2) inhibition or retardation of cancer in individuals with pre-malignant lesions or; (3) secondary prevention or inhibition of cancer recurrence in patients already having treatment for a primary cancer.[4] Many studies have been reported on mechanisms for chemoprevention, especially prevention of carcinogenesis.[5,6] Among them, detoxification of toxic quinones by quinone reductase (QR, also called NQO1) is one of recognized mechanisms for chemoprevention. Quinone reductase, a representative phase II detoxification enzyme, is revealed to have a close relationship with prevention of cancer by blocking cancer initiation. Thus, QR induction is used as a biomarker of chemopreventive activities and their potency.[7,8]

Up to date, various natural compounds with chemopreventive effects were isolated mostly from dietary plants, and most of them were reported to show their activities by preventing carcinogenesis. Some representative agents include sulforaphane from cruciferous vegetables, lycopene from tomato products, and resveratrol from grapes.[9,10] In particular, oltipraz, a dithiolethione class chemopreventive agent, has evaluated its anti-cancer activity in phase II clinical trial, though it failed due to the low efficacy and side effects.[11]

In previous publications, we reported the isolation of gymnasterkoreaynes B, E, and G from *Gymnaster koraiensis* by activity-guided fractionations and their potent cancer chemopreventive effects.[12] These compounds induced phase II detoxification enzymes known to have cytoprotective functions, such as glutathione-*S*-transferase, UDP-glucuronosyltransferase, NAD(P)H: quinone oxidoreductase (NQO1), and glutathione reductase (GSR), in normal and HepG2 human hepatocarcinoma cells. The gymnasterkoreaynes are naturally occurring polyacetylenic compounds. Our group recently completed the total synthesis of gymnasterkoreaynes E and G, which contain octa-4,6-diyne-2,3,8-triol as a major functional backbone (Fig. 1).[13] We also described the structure–activity relationship (SAR) of these polyacetylenes on cancer chemopreventive activity.[14] In that study, important structural information regarding the use of diyne triols in cancer chemoprevention was revealed: the reduction of the diyne moiety to a saturated alkyl group fully eliminated both chemopreventive activity and cytotoxicity, and the variations of the terminal alkyl groups had significant effects on the induction potency of quinone reductase and cellular toxicity.

Structurally, gymnasterkoreaynes G, E and B have *cis*-olefin and three stereocenters. Such molecular complexities limited the synthesis of optically pure form of active stereoisomers and also the production of a large amount of active compounds for in vivo study. Hence, on the basis of the established SAR, we hypothesized

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http://dx.doi.org/10.1016/j.bmcl.2015.05.098
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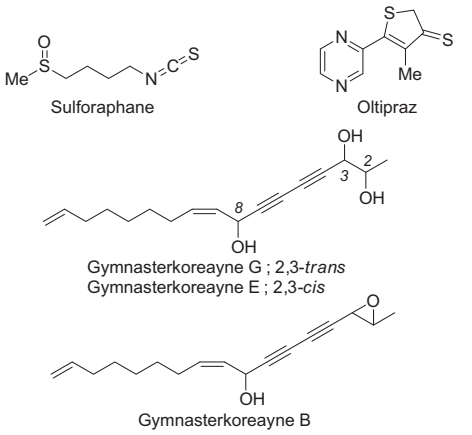


Figure 1. Structures of gymnasterkoreayne E, G, and B.

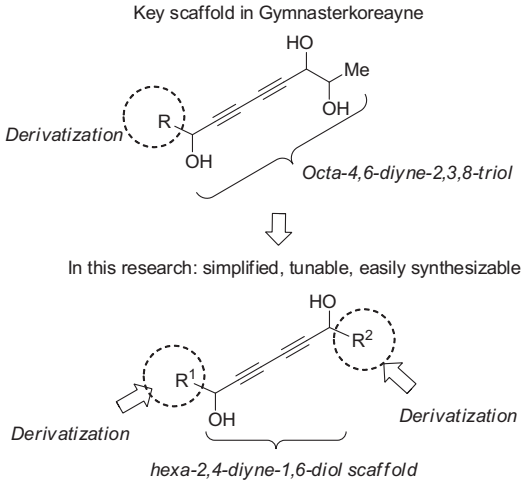
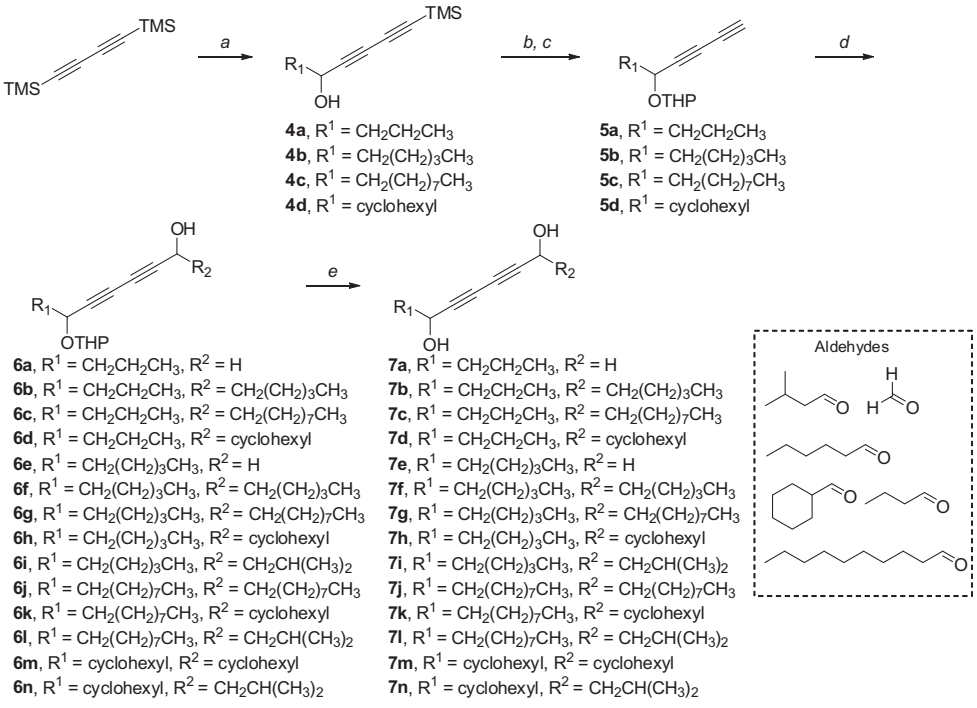


Figure 2. Design of diyne diols based on diyne triols.

that a structurally simplified diyne-based scaffold which retains diacetylenic diol moiety might have similar biological activities and is much easier to synthesize as stereoisomeric mixtures or single stereoisomers than diacetylenic triols. Herein, we report the synthesis and cancer chemopreventive activities of new diyne diols and eventually, creation of compounds for in vivo study (Fig. 2).

Syntheses of the diacetylene diol analogues were conducted using the previously established synthetic procedure by our group, as illustrated in Scheme 1. Starting from the commercially available 1,4-bis(trimethylsilyl)buta-1,3-diyne, various alkyl aldehydes were installed on both ends of 1,3-butadiyne. Generation of acetylenic anions from 1,4-bis(trimethylsilyl)buta-1,3-diyne through a metal-silicon exchange reaction by treating methyl lithium–lithium bromide in THF, followed by the addition of alkyl aldehyde produced acetylenic alcohols **4a**–**4d** in excellent yields.[15] Sequential protection of the secondary alcohol with THP and desilylation of terminal trimethylsilyl by TBAF gave compounds **5a**–**5d**. In the THP protection step, two diastereomeric mixtures were detected in thin-layer chromatography and were barely separable, and so both diastereomers were used for the next step without separation. Terminal alkynes were deprotonated by ethylmagnesium bromide and treated with alkyl aldehydes to give THP-protected dialkyl hexa-2,4-diyne-1,6-diols **6a**–**6n**. Finally, deprotection of THP under the condition of a 9:1 mixture of 70% AcOH in H_2O and THF gave the desired diols **7a**–**7n**. After synthesis of the derivatives, we recognized that **7b**, **7e**, **7f**, **7j**, and **7m** had been previously reported.[16–20] Overall, 14 diyne diols were synthesized by modification of both terminals with different alkyl groups. By fixing the left terminal with *n*-propyl, *n*-pentyl, *n*-nonyl, or cyclohexyl groups, the right terminal was derivatized with other alkyl groups.

We exploited the potency of cancer chemopreventive activity induced by dialkyl diacetylene diols by measuring the quinone reductase (QR) assay in Hepa1c1c7 murine hepatoma cells. The QR assay, a useful tool for the evaluation of cancer chemopreventive activity, was performed according to the Prochaska modified method.[8,21] The potency of the cancer chemopreventive activity



4a, $R^1 = CH_2CH_2CH_3$
4b, $R^1 = CH_2(CH_2)_3CH_3$
4c, $R^1 = CH_2(CH_2)_7CH_3$
4d, R^1 = cyclohexyl

5a, $R^1 = CH_2CH_2CH_3$
5b, $R^1 = CH_2(CH_2)_3CH_3$
5c, $R^1 = CH_2(CH_2)_7CH_3$
5d, R^1 = cyclohexyl

6a, $R^1 = CH_2CH_2CH_3$, R^2 = H
6b, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
6c, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
6d, $R^1 = CH_2CH_2CH_3$, R^2 = cyclohexyl
6e, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = H
6f, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
6g, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
6h, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = cyclohexyl
6i, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2CH(CH_3)_2$
6j, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
6k, $R^1 = CH_2(CH_2)_7CH_3$, R^2 = cyclohexyl
6l, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2CH(CH_3)_2$
6m, R^1 = cyclohexyl, R^2 = cyclohexyl
6n, R^1 = cyclohexyl, $R^2 = CH_2CH(CH_3)_2$

7a, $R^1 = CH_2CH_2CH_3$, R^2 = H
7b, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
7c, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
7d, $R^1 = CH_2CH_2CH_3$, R^2 = cyclohexyl
7e, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = H
7f, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
7g, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
7h, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = cyclohexyl
7i, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2CH(CH_3)_2$
7j, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
7k, $R^1 = CH_2(CH_2)_7CH_3$, R^2 = cyclohexyl
7l, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2CH(CH_3)_2$
7m, R^1 = cyclohexyl, R^2 = cyclohexyl
7n, R^1 = cyclohexyl, $R^2 = CH_2CH(CH_3)_2$

Scheme 1. Synthesis of diyne diols. Reagents and conditions: (a) MeLi–LiBr, THF, 0 °C to rt; then aldehyde, 78–83%; (b) DHP, *p*-TsOH, CH_2Cl_2, rt, 77–88%; (c) TBAF, THF, rt, 82–87%; (d) EtMgBr, 0 °C to rt; then aldehyde, 67–78%; (e) 70% AcOH in H_2O/THF (9:1), 40 °C, 71–83%.

Table 1
Cancer chemopreventive activity of diols

Compound	R^1	R^2	CD (μM)*	IC_{50} (μM)*	CI
7a	*n*-Propyl	H	6.0 (±0.7)	>50	>8.3
7b	*n*-Propyl	*n*-Pentyl	0.8 (±0.5)	19 (±13.3)	24
7c	*n*-Propyl	*n*-Nonyl	4.2 (±2.1)	30 (±6.4)	7.2
7d	*n*-Propyl	Cyclohexyl	6.2 (±0.6)	>50	>8.1
7e	*n*-Pentyl	H	4.7 (±2.1)	>50	>11
7f	*n*-Pentyl	*n*-Pentyl	3.1 (±4.5)	>50	>16
7g	*n*-Pentyl	*n*-Nonyl	2.6 (±0.7)	12 (±4.2)	4.6
7h	*n*-Pentyl	Cyclohexyl	3.2 (±1.5)	>50	>16
7i	*n*-Pentyl	2-Methylpropyl	1.3 (±0.2)	41 (±9.0)	32
7j	*n*-Nonyl	*n*-Nonyl	26 (±4.5)	26 (±3.7)	1.0
7k	*n*-Nonyl	Cyclohexyl	11 (±0.5)	44 (±2.0)	4.0
7l	*n*-Nonyl	2-Methylpropyl	10 (±1.3)	>50	>5.0
7m	Cyclohexyl	Cyclohexyl	8.4 (±3.1)	>50	>6.0
7n	Cyclohexyl	2-Methylpropyl	5.6 (±2.3)	>50	>8.9
Gymnasterkoreayne G			8.2 (±3.3)	>50	>6.1
SFN			0.5	15 (±0.5)	27

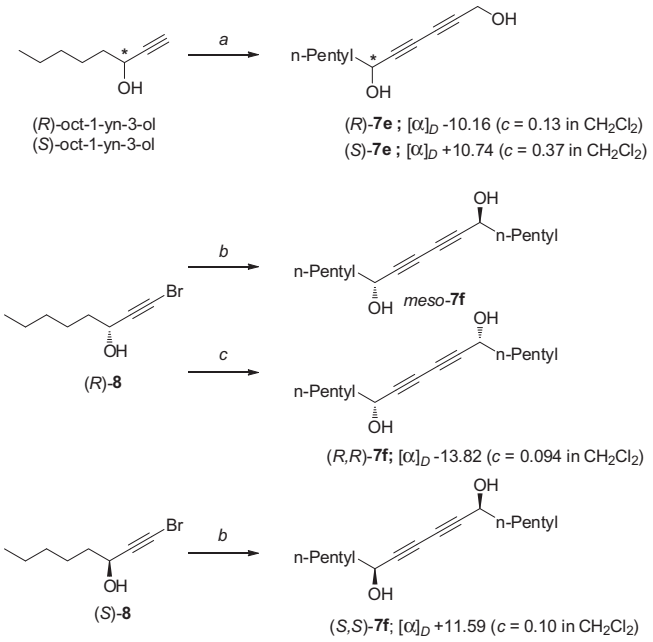
* CD and IC_{50} values represent the mean ± SD, $n = 6$.

was expressed as a chemoprevention index (CI), calculated by dividing the concentration that inhibited 50% of the cell proliferation (IC_{50}) by the concentration required to double the QR activity (CD value). Sulforaphane (SFN) and gymnasterkoreayne G were used as positive controls.

As shown in Table 1, chemopreventive activities of the synthesized compounds which most of the compounds presented by chemopreventive index (CI) values. Among the tested dialkyl diacetylene diols, 11 derivatives displayed good activities with CD of 0.8–8.4 μM and just three compounds exhibited two digit micromolar activity. In particular, **7b** displayed the best cancer CD activity (CD = 0.8) which is much better than gymnasterkoreayne G (CD = 8.2) and almost equipotent with sulforaphane (SFN; CD = 0.5). Another advantage of dialkyl diacetylene diols is their low cytotoxicity. All compounds except **7g** showed lower toxicity than sulforaphane and **7a**, **7d**–**7f**, **7h**, and **7l**–**7n** exhibited no cytotoxicity under 50 μM. One tendency is that every nonyl derivative except **7l** showed mild cytotoxicity and long alkyl chain diminished the CD value. Considering both biological activity and safety, **7e**, **7f**, and **7h** were thought to be optimal for in vivo study (CD = 4.7, 3.1, 3.2 μM; IC_{50} = >50, >50, >50 μM; CI = >11, >16, >16, respectively), although **7b** and **7e** were slightly better in terms of CD activity (CD = 0.8 and 1.3 μM, respectively).

In the early stage of drug discovery, compounds with one or more chiral carbon are often used for assays as stereoisomeric mixtures, and then biologically active compounds are requested to prove which optical stereoisomers exert influence on its activity or toxicity. In this study, selected **7e**, **7f**, and **7h** are stereoisomeric mixtures; **7e** is racemates and **7f** and **7h** are diastereomeric mixtures. Considering synthetic feasibility as well as biological activity, we carried out synthesis of all stereoisomers of **7e** and **7f** as optically pure form in order to address the effect of stereochemistry on their chemopreventive activity and toxicity, and eventually to make a final selection of compounds for in vivo animal study. Synthesis of the compounds (*R*)-**7e** and (*S*)-**7e** was accomplished by copper-mediated cross coupling of commercially available (*R*) and (*S*)-oct-1-yn-3-ol with 3-bromoprop-2-yn-1-ol.[22] In case of **7f**, there exist three stereoisomers, (*R,R*)-**7f**, (*R,S*)-**7f**, and *meso*-**7f** due to the existence of a symmetry plane of (*R,S*)-**7f**. As shown in Scheme 2, bromoalkyne (*R*)-**8** prepared from (*R*)-oct-1-yn-3-ol was coupled with (*S*)-oct-1-yn-3-ol and (*R*)-oct-1-yn-3-ol to afford *meso*-**7f** and (*R,R*)-**7f**, respectively. In th same way, (*S,S*)-**7f** was also synthesized by coupling of (*S*)-**8** and (*S*)-oct-1-yn-3-ol.

Quinone reductase induction activity, cell viability, and CI values of the synthesized optically pure compounds are presented in Table 2. Both of the (*R*)-**7e** and (*S*)-**7e** showed similar biological



Scheme 2. Synthesis of optically pure stereoisomers of **7e** and **7f**. Reagents and conditions: (a) 3-bromoprop-2-yn-1-ol, $CuCl_2$, NH_2OH–HCl, $EtNH_2$, MeOH, rt, 37%; (b) (*S*)-oct-1-yn-3-ol, $CuCl_2$, NH_2OH–HCl, $EtNH_2$, MeOH, rt, 42–52%; (c) (*R*)-oct-1-yn-3-ol, $CuCl_2$, NH_2OH–HCl, $EtNH_2$, MeOH, rt, 46%.

Table 2
Cancer chemopreventive activities of chiral diols

	CD (μM)	IC_{50} (μM)	CI
(*R*)-**7e**	2.3 (±1.7)	>100	>43
(*S*)-**7e**	3.2 (±0.8)	>100	>31
meso-**7f**	4.7 (±2.1)	68 (±3.2)	14
(*R,R*)-**7f**	0.6 (±2.8)	12 (±2.3)	20
(*S,S*)-**7f**	2.6 (±1.9)	47 (±4.6)	18
SFN	0.4	11	28

profiles with (±)-7**e** in terms of CD activity and cellular toxicity. The stereochemistry of chiral carbon in 7**e** did not affect their biological activities. The better is both stereoisomers did not display cytotoxicity under 100 μM. In case of **7f**, chemopreventive activities were slightly different depending on the stereochemistry. (*R,R*)-**7f** exhibited most potent quinone reductase induction effect

and cytotoxicity (CD = 0.6 and IC_{50} = 12 μM), which was almost equal to sulforaphane (CD = 0.4 and IC_{50} = 11 μM), while *meso*-**7f** and (*S,S*)-**7f** displayed comparable activities. In this case, all stereoisomers retained their CD and cytotoxic activities, but were sensitive to stereochemistry. Overall, stereochemistry seems not to have vital influence on biological activities.

In summary, we discovered novel cancer chemopreventive agents with a hexa-2,4-diyne-1,6-diol scaffold. Diyne diols were designed based on gymnasterkoreaynes E and G, naturally occurring diyne triols with cancer chemopreventive activity. The diols were readily obtained using a five-step procedure that was much easier than that of the corresponding triols. Most diols exhibited good biological activity in terms of quinone reductase induction, cell viability, and CI. In particular, as diastereomeric mixtures, **7b** and **7i** showed the best cancer chemopreventive activity, which was nearly equipotent to that of SFN, and **7e**, **7f**, and **7h** were considered to be optimal in terms of safety as well as activity. And, we synthesized full series of stereoisomers of **7e** and **7f** as optically pure forms from chiral building blocks and evaluated their chemopreventive activity. The stereochemistry affected their biological activities to some extent, but it seems not to be crucial.

Acknowledgements

This work was supported by Basic Science Research Program through the National Research Foundation of South Korea (NRF) funded by the Ministry of Education, Science and Technology of South Korea (NRF-2012R1A1A1007057) and partly by KIST intramural grant (2Z03850).

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Further reading

23. *Spectral data of selected compounds;* ***7e****:* Colorless oil; R_f = 0.23 (EtOAc/hexanes, 1:3); ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.44 (dd, J = 6.0, 6.6 Hz, 1H), 4.35 (m, 2H), 1.79 (d, J = 6.0 Hz, 1H), 1.76–1.69 (m, 2H), 1.6 (t, J = 6.6 Hz, 1H), 1.47–1.42 (m, 2H), 1.34–1.30 (m, 4H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 80.6, 77.5, 69.9, 68.8, 62.9, 51.5, 37.5, 31.4, 24.7, 22.5, 14.0; FT-IR ν_{max} 3315.3, 2923.4, 2854.8, 1459.1, 1335.2, 1020.0; MS (ESI) $[M+H]^+$ 181; **7f**: Colorless oil; R_f = 0.3 (EtOAc/hexanes, 1:3); ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.43 (t, J = 5.7 Hz, 2H), 1.81 (s, 1H), 1.72 (m, 4H), 1.45 (m, 4H), 1.32 (m, 8H), 1.90 (t, J = 6.9 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 80.5, 68.9, 62.9, 37.5, 31.4, 24.7, 22.5, 14.0;FT-IR ν_{max} 331.4, 2927.5, 2859.1, 2360.6, 2341.2, 1458.5, 1020.4; MS (ESI) $[M+H]^+$ 251.