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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 3525–3528

Synthesis of a tetra-deuterium-labeled derivative of potent and selective anticancer agent AA005

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Received 28 January 2005; revised 10 March 2005; accepted 14 March 2005
Available online 6 April 2005

Abstract—Annonaceous acetogenins are a series of potent naturally occurring anticancer agents, which act as inhibitors of complex I in mitochondria. AA005, a mimicry of acetogenins, has been found as active as those natural products and to present high selectivities between cancer and normal cells. In order to investigate the further cell-based mechanism induced by AA005, a d^4-labeled derivative of AA005 (AA005-d^4) was designed to detect the drug permeation ability into the membranes. In this letter, the synthesis is reported of this deuterium-labeled compound, wherein a ethylene-d^4 glycol unit is incorporated efficiently into the molecule skeleton by simple etherifications.

1. Introduction

Annonaceous acetogenins, a family of naturally occurring polyketides, are well known for their cytotoxic and anti-tumoral activities.[1,2] They were considered to be the most powerful inhibitors of complex I (NADH-ubiquinone oxidoreductase) in mitochondria.[3] Because of the lack of detailed structural information about complex I, there is still no clear experimental evidence to elucidate its binding sites. In our recent work, we focused on structural modifications on the basis of natural structures and developed a series of annonaceous acetogenin mimetics.[4–9] These mimics have been found to be as potent as those active natural products, and some of which even showed highly distinctive abilities to only kill the cancer cells and not to affect the normal cells. Among them, AA005 (**1** in Fig. 1) is a representative with IC_{50} ranges from 50 nM to 100 nM against a variety of cancer cells.[5] AA005 is significantly simplified from one of natural acetogenins, bullatacin (Fig. 1), by deleting four carbons from the THF region of its natural counterpart. Recent biological evidences show that it efficiently induces the cancer cells death in 48 h, and no action to the normal cells.[7] Such selectivities caused by AA005 are rarely reported, and therefore, the further mechanism study is of great value for future drug

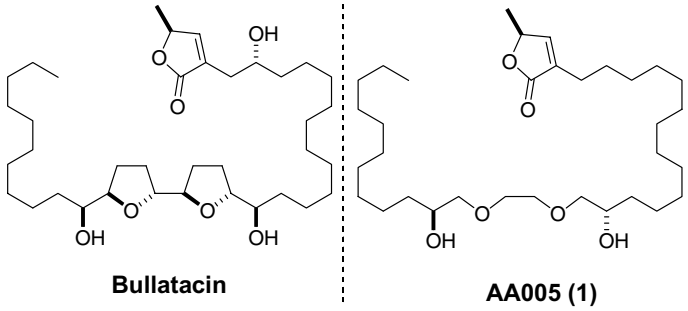

Figure 1. Bullatacin and its typical mimicry AA005.

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doi:10.1016/j.tetlet.2005.03.123

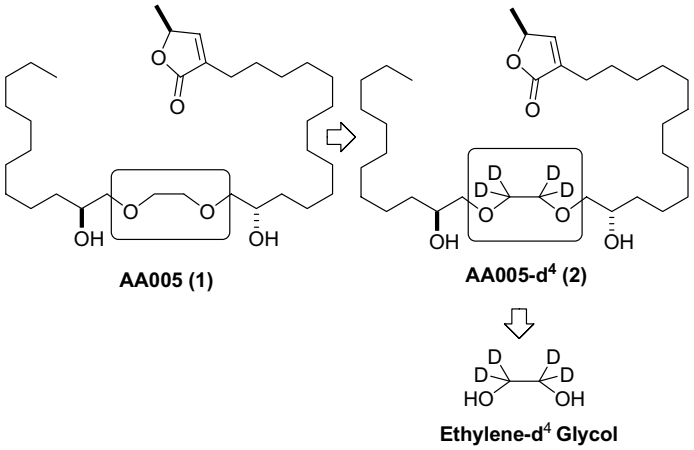


Figure 2. Stable isotope labeled derivative of AA005.

design. In other words, AA005 is a valuable biological tool to probe those related unknown or unclear mechanisms, by which cancer cell death is induced in a selective way. Membrane selectivity between normal cells and tumor cells by AA005 is the first question we want to know. A direct shortcut for this question is to measure the concentration difference of AA005 inside different cells. Considering the low abundance of AA005 in cells, mass spectrometry-based methodology is chosen to meet the sensitivity requirements. In order to acquire the more clear and accurate information from complex background in cell, a proper stable isotope labeled derivative of AA005 becomes a must.

Stable isotope labelings with ^{13}C, ^{15}N, ^{18}O, or deuterium[10] are used commonly in quantification in mass spectrometry. In order to make the quantification of AA005 in different cells possible, we design a stable isotope labeled AA005 (heavy reagent), which will be administrated with its light counterpart AA005, both in cancer cells and normal cells in equal quantity. The deuteriums in the deuterium-labeled molecule AA005-d^4 should be introduced by an easy way and located stably. Figure 2 shows our considerations for its synthesis. A commercially available agent, ethylene-d^4 glycol is chosen as the labeled building block, which will be incorporated into the chemically inert ether region of AA005.

2. Results and discussion

Retrosynthetic analysis of AA005-d^4 **2** is illustrated in Figure 3. Successive *O*-alkylations of ethylene-d^4 glycol with (*R*)-(−)-epichlorohydrin **13** and **11**, respectively, are designed as the key steps to introduce the four deuteriums, and functionalize the middle region of AA005-d^4 in the mean time. Trimethylsilylacetylene **7** plays as a two-directional *C*-alkylation reagent to link both epoxide-intermediates **8** and **6** together, constructing the whole molecular skeleton of AA005-d^4. The starting chiral materials **14**, **13**, and **9** are readily available or easy to prepare.

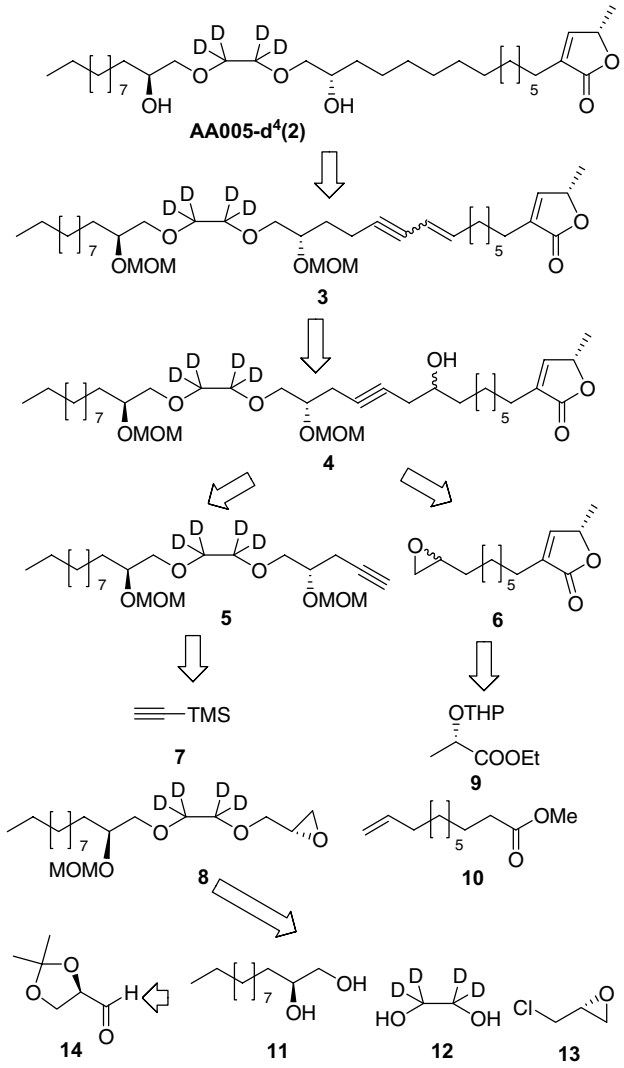
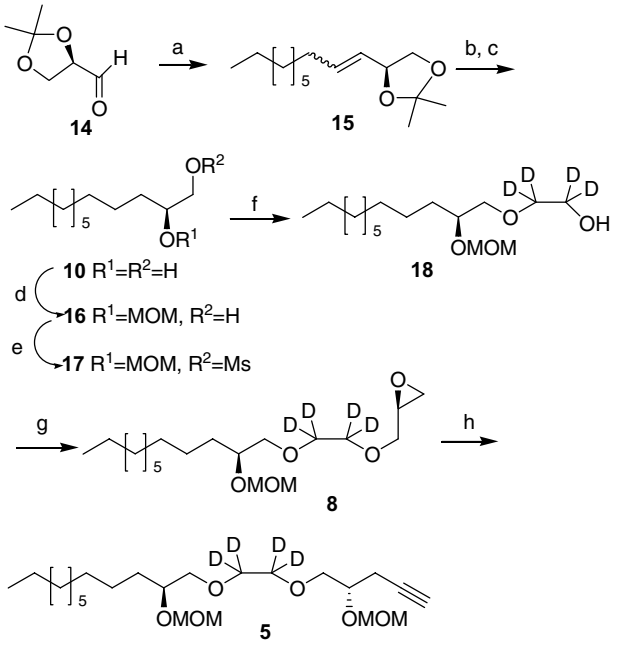


Figure 3. Retro synthetic analysis of AA005-d^4.

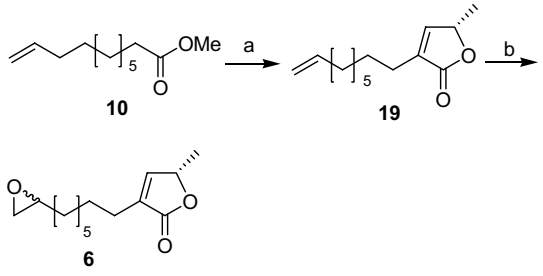
Key intermediate **5** is synthesized as shown in Scheme 1. Starting from chiral aldehyde **14**, a three-step reaction sequence was applied to obtain the diol **10**.[6,11] The resul-



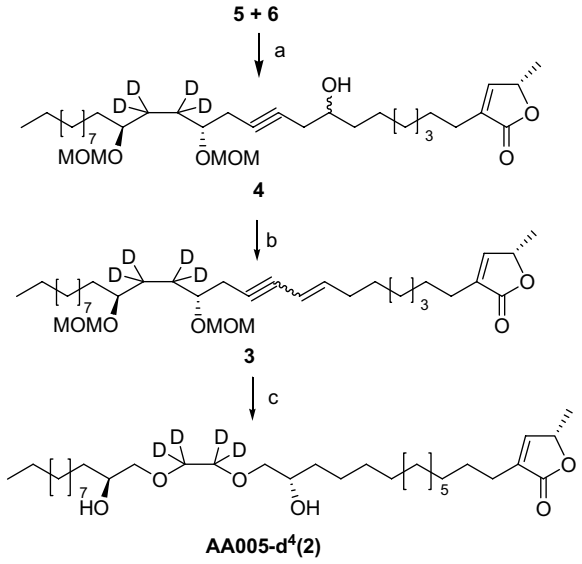
Scheme 1. Reagents and conditions: (a) $C_8H_{17}CH{=}PPh_3$, 90%; (b) 10% Pd–C, H_2, CH_3OH; (c) 10% HCl, CH_3OH, 88% over two-steps; (d) (i) $HC(OCH_3)_3$, D-10-CSA, CH_2Cl_2, (ii) DIBALH in toluene, CH_2Cl_2, 0 °C, 90% over two-steps; (e) MsCl, Et_3N, CH_2Cl_2, 100%; (f) NaH, DMF, 130 °C, 57%; (g) NaOH, H_2O, Bu_4NHSO_4, (*R*)-epichlorohydrin, 79%; (h) (i) *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, trimethylsilylacetylene, −78 °C; (ii) *i*-Pr_2NEt, MOMCl, CH_2Cl_2; (iii) TBAF, THF, 0 °C, 76% over three-steps.

tant diol **10** was protected with MOMCl regioselectively to afford **16** (90%) by a two-step procedure.[9,12] The remaining primary hydroxyl group was then masked as a mesylate (**17**, 100% yield). The following reaction of mesylate **17** with ethylene-d^4 glycol **11** was carried out in the presence of NaH in DMF, giving **18** in 57% yield. Coupling of alcohol **18** with (*R*)-(−)-epichlorohydrin **13** was achieved in the presence of a phase-transfer catalyst and furnished **8** in 88% yield.[13] The following regioselective opening of epoxides **8** with trimethylsilyl acetylene mono-lithium salt, MOM-protection, and TMS-deprotection elaborated the left part **5**.[14]

The right part **6** was synthesized as shown in Scheme 2. Subsequent introduction of the butenolide unit to **8**



Scheme 2. Reagents and conditions: (a) (i) LDA, THF–HMPA, (*S*)-*O*-tetrahydropyranyl lactal, −78 °C; (ii) 10% H_2SO_4, THF, rt; (iii) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 60% over three-steps; (b) *m*-CPBA, CH_2Cl_2, 0 °C, 86%.



Scheme 3. Reagents and conditions: (a) *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, −78 °C, 94%; (b) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C ; (ii) DBU, THF, rt, 43%; (c) (i) $TsNHNH_2$, NaOAc, DME, reflux; (ii) concd HCl, MeOH, 46% over two-steps.

involved a three-step sequence[4,15] previously developed by us: (i) aldol reaction of **10** with freshly prepared (2*S*)-*O*-tetrahydropyranyl lactal; (ii) acid-catalyzed THP cleavage and in situ lactonization; and (iii) β-elimination of hydroxyl in the presence of $(CF_3CO)_2O$ and Et_3N. Regioselective epoxidation of **19** was achieved by *m*-CPBA to give **6** in 86% yield.

With both functionalized synthons **5** and **6** in hand, construction of the whole skeleton was performed as follows (Scheme 3). Treatment of terminal alkyne **5** with butyllithium followed by reaction with **6** in the presence of BF_3 etherate at −78 °C gave whole skeleton **4** in 70% yield. Elimination of newly formed hydroxyl group in the presence of MsCl–Et_3N and then DBU afforded **3**. Selective reductions of triple and double bonds in the middle region of **3** were carried out by diimide-based reductions.[14b] Final deprotection of MOM ethers was accomplished in MeOH with catalytic amount of concentrated HCl, affording final product AA005-d^4 **2**. All the physical data[16] were excellent agreement with the structure of AA005-d^4.

3. Conclusion

In summary, AA005-d^4, a stable isotope-labeled derivative of anticancer agent AA005 was synthesized enantioselectively. This is the first example of annonaceous acetogenin mimicry containing multiple deuteriums in the designed location. The reported synthesis presents advantages of high efficiency and use of readily available reagents and materials. The cell membrane-penetration studies using AA005 and AA005-d^4 by mass spectrometry is currently underway, and result will be reported in due course.

Acknowledgements

This work was financially supported by the Major State Basic Research and Development Program (G2000077500), NSFC (30271531, 20425205, and 20321202), the Chinese Academy of Sciences (KGCX2-SW-209) and Shanghai Municipal Commission of Science and Technology (04DZ14901).

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16. Physical data for AA005-d^4 (**2**): $[\alpha]_D^{25}$ +7.22 (*c* 0.64, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): 0.88 (3H, t, J = 6.6 Hz), 1.10–1.60 (40H, m), 1.41 (3H, d, J = 6.6 Hz), 2.27 (2H, t, J = 7.8 Hz), 2.99 (2H, br) 3.16 (t, 2H, J = 5.7 Hz), 3.53 (2H, d, J = 6.9 Hz), 3.78 (2H, m), 5.00 (1H, qd, J = 6.9, 1.5 Hz), 7.00 (1H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 173.9, 148.9, 134.3, 76.6, 75.8, 70.3, 33.0, 31.9, 29.7, 29.6, 29.6, 29.5, 29.3, 29.3, 29.2, 27.4, 25.6, 25.2, 22.7, 19.2, 14.1 ppm. IR (film): 3491, 2914, 2849, 1749, 1474, 1314, 1124 cm^{-1}. MS (ESI, *m/z*): 581 (M^++Na). HRMS (ESI) calcd for $C_{33}H_{58}D_4O_6Na$ [M^++Na]: 581.4689. Found 581.4703.