Asymmetric Total Synthesis of (+)- and (−)-Clusianone and (+)- and (−)Clusianone Methyl Enol Ether via ACC Alkylation and Evaluation of their Anti-HIV Activity

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Abstract

The total asymmetric synthesis of (+)- and (−)-clusianone and (+)- and (−)-clusianone methyl enol ether is reported. Asymmetric induction is achieved through the use of ACC alkylation, providing the key intermediates with an *er* of 99:1. The four synthetic compounds were evaluated for their anti-HIV activity. Both (+)- and (−)-clusianone displayed significant anti-HIV activity.

Keywords

ACC ketone alkylation; Asymmetric synthesis; Polycyclic polyprenylated acylphloroglucinols; Viral infection; Anti-HIV activity

Viral infections are a pervasive form of disease that range from the common cold to much more deadly forms such as AIDS. As with antibiotics,[1] current antiviral therapies are becoming less effective due to the development of resistant strains.[2] Consequently, there is an urgent need for new anti-viral agents. (+)-Clusianone (**1**) (Figure 1) is a member of a large class of biologically active natural products termed the polycyclic polyprenylated acylphloroglucinols (PPAPs).[3] (+)-Clusianone has been shown to possess antiviral activity against both HIV ($EC_{50} = 0.020 \pm 0.003$ μM),[4] and Epstein-Barr virus ($17.4 \pm 1.2\%$ of cells were EBV-EA positive in the presence of 32 nmol of **1**).[5] As such, it is a compelling target for further biological investigation as an antiviral agent. Unfortunately, no asymmetric total synthesis is available, as is necessary for further biological testing and medicinal chemistry efforts.

Not only do PPAPs generally exhibit biological activity, but they also possess intriguing structures.[3] Consequently, they have attracted considerable interest from the synthetic community. Despite the impressive advances culminating from the synthetic work carried out to date on the PPAPs, asymmetric total syntheses are rare.[6] In the case of clusianone,

Supplementary Material
Experimental procedures and analytical data for all new compounds is available.

total syntheses have been reported by Danishefsky,[7] Simpkins,[8] Porco,[9] and Marazano.[10] However, only one synthesis of optically active (+)-clusianone has been reported,[11] and this was achieved via chiral resolution of a late-stage synthetic intermediate.

We recently described a new method for the asymmetric α-alkation of ketones utilizing chiral *N*-amino cyclic carbamate (ACC) auxiliaries (cf. **9** and **10**, Scheme 1).[12] ACC auxiliaries react readily with ketones to afford the corresponding hydrazones (**5 → 6**). These undergo rapid deprotonation to the azaenolates, which can be alkylated on up to a multi-gram scale with excellent stereoselectivity and yield (**6 → 7**). Moreover, the auxiliaries can be recovered quantitatively and recycled. We realized that this enantio-selective alkylation method could provide the basis for an efficient asymmetric total synthesis of the clusianones. In an earlier communication,[13] we described the first asymmetric total synthesis of (+)-clusianone (**1**) and its methyl enol ether (**3**). In what follows we describe the extension of this synthetic strategy to the first asymmetric total synthesis of (−)-clusianone (**2**) and its methyl enol ether (**4**). Whereas some PPAPs have been isolated in both their (+)- and (−)-forms, clusianone has not, so this constitutes the first report of (−)clusianone. We also report our initial biological investigation of compounds **1**–**4** with regard to their anti-HIV activity. To our knowledge, this is the first instance in which the two enantiomers of a PPAP have been tested in the same biological assay.

Our general retrosynthetic plan is summarized in Scheme 2 by reference to (−)-clusianone (**2**), and is analogous to that used previously by us for the synthesis of (+)-Clusianone (**1**).[13] We would engage optically pure enol ether **12** in a diastereoselective Effenberger cyclization[14] to generate the advanced bicyclic intermediate **11**, as done by Simpkins in his synthesis of racemic clusianone.[8a] This would be elaborated to optically pure **2** using established procedures.[8a] Conjugate addition and enol ether formation would provide access to **12** from **13**. The preparation of **13** would be effected by 1,2-methylation of α,β-unsaturated ketone **14**, followed by a Babler-Dauben[15] oxidation of the resulting tertiary allylic alcohol. Synthesis of the key optically pure intermediate **14** would be achieved via enantioselective ACC alkylation,[12] beginning from known enone **15**.

We began our studies by preparing enone **15** using a combination of literature procedures,[10,16,17] which was then subjected to enantioselective ACC alkylation. To begin this process, compound **15** was converted to hydrazone **16** by acid mediated condensation with ACC auxiliary **9**.[18] Once formed, **16** was subjected to LDA-mediated prenylation, giving **17** in excellent yield, and as a single diastereomer, as judged by ^{1}H and ^{13}C NMR analysis. Auxiliary removal from prenylated hydrazone **17** was achieved hydrolytically by treatment with *p*-TsOH·H$_2$O in acetone/H$_2$O, producing the key synthetic intermediate **14** in 80% yield. Analysis of synthetic **14** by chiral HPLC revealed an enantiomer ratio of 99:1 indicating that minimal, if any, epimerization occurred during removal of the ACC auxiliary.

With optically active ketone **14** in hand, we initiated the methylation/carbonyl transposition sequence. Treatment of **14** with the Grignard reagent MeMgBr efficiently produced tertiary allylic alcohol **18**. This underwent Babler-Dauben oxidation upon exposure to PCC producing enone **13**, marking a convergence point with Simpkins' synthesis of racemic clusianone.[8b] α,β-Unsaturated ketone **13** was, therefore, subjected to conjugate addition using a methyl cuprate to produce **19**. The methyl enol ethers **12** and **20** were generated from **19** upon treatement with *t*-BuOK and Me$_2$SO$_4$, setting the stage for the Effenberger cyclization. Careful acid/base extraction was required following this transformation to separate the desired product (**11**) from **19**, and the latter was able to be recycled to produce more of **12** and **20**. Methylation of crude cyclized product **11** gave **21** in 18% yield over the two steps (35% based on recovered **19**). Next, **21** was subjected to bridgehead prenylation, which gave **22** in excellent yield. Subsequent addition of the benzoyl group gave rise to (+)-

clusianone methyl enol ether (**4**). Finally, simple hydrolysis was used to afford (−)-clusianone (**2**).

(+)-Clusianone (**1**) and the corresponding (−)-clusianone methyl enol ether (**3**) were prepared from ketone **15**, but using the (*R*)-benzyl-2-oxazolidinone-derived ACC auxiliary.[13]

To determine the enantiomeric purity of our synthetic material, the above synthetic sequence was carried out in a racemic fashion to the stage of the penultimate compound.[13] Thus, enone **15** underwent LDA-mediated prenylation, producing (±)-**14**. This compound was then subjected to the transformations developed above to give a racemic mixture of **3** and **4**. Conditions were established that gave baseline resolution of **3** and **4** by chiral HPLC. Subsequent analysis of synthetic **3** produced via asymmetric ACC alkylation (Scheme 3) revealed an *er* = 99:1. Similar analysis of synthetic **4** gave an equally high *er*.[13]

Having completed the asymmetric total synthesis of compounds **1–4**, we undertook an investigation into their anti-HIV activity. To do so, we created HIV-1 Env-psuedoviruses and tested the ability of compounds **1–4** to block their infectivity in a mammalian cell culture system. Pseudoviruses were made according to the methods of Montefiori et al.[19] Briefly, 293T-cells were co-transfected with an env-deficient HIV-1 genome that contains a firefly luciferase gene inserted into the HIV nef gene (pNLCH5.1)[20] and a second plasmid containing an envelope gene (pNL4.3 [CXCR4-tropic] or pJRFL [CCR5-tropic], both obtained from the AIDS Reagent Repository). Target cells for these HIV-1 Env-pseudoviruses were 3T3.T4.CCR5 and Jurkat E6-1 cells, both of which were obtained from the NIAID AIDS Reference Program. 3T3 cells were incubated with a 1:4 dilution of the pJRFL HIV-1 Envpseudoviruses and Jurakat E6-1 cells were incubated with a 1:4 dilution of the pNL4.3 HIV-1 Env-pseudoviruses. When the Env-psuedoviruses successfully infect the target cells, the viral genome becomes integrated into the cellular genome and luciferase is produced. Luciferase activity was measured with the Promega GloMax system according to the manufacturers instructions. To test the ability of compounds **1–4** to inhibit viral infectivity, the compounds were serially diluted 3-fold from 10 μM to 4.6 nM, all at a final concentration of 1% DMSO. All infections were carried out in duplicate; the 3T3 results are representative of three experiments and the Jurkat results are from one experiment. IC_{50} values were calculated with Graphpad Prism.

Interestingly, despite being enantiomers of one another, (+)- and (−)-clusianone (**1 and 2**) exhibited very similar IC_{50} values of 1.53 and 1.13 μM, respectfully, in the 3T3 system.[21] However, compounds **3** and **4** showed no inhibition (Figure 2A). Similar results were obtained in the Jurkat cells, although the IC_{50} of compounds **1** and **2** were lower than the IC_{50} observed in the 3T3 cells (Figure 2B). The IC_{50} values of the clusianone compounds are higher than the reported IC_{50} values for AZT (~120 nM)[22] and nevirapine (~40 nM).[23] As mentioned above, some PPAPs have been isolated in both their (+)- and (−)- forms. These include the enantiomeric pairs hyperibone G[24] and propolone D,[25] hyperibone A[24] and garcinielliptone I,[26] guttiferone E[27–30] and carcinol,[28] and isoxanthochymol[27,28] and isogarcinol.[31] However, this appears to be the first instance in which an enantiomeric pair of PPAPs has been evaluated in the same biological assay.

In conclusion, (+)- and (−)-clusianone and (+)- and (−)-clusianone methyl enol ether have been synthesized in a highly enantioselective (*er* = 99:1) fashion using asymmetric ACC alkylation. Biological analysis of these compounds was conducted to determine their potential anti-HIV activity. While the degree of antiviral activity depended on cell type, both (+)- and (−)-clusianone (**1** and **2**, respectively) displayed significant anti-HIV activity.

Despite their enantiomer relationship, compounds **1** and **2** exhibited essentially the same inhibitory activity. The mechanism of this activity is currently unknown.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References and notes

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17. See the Supporting Information for details.

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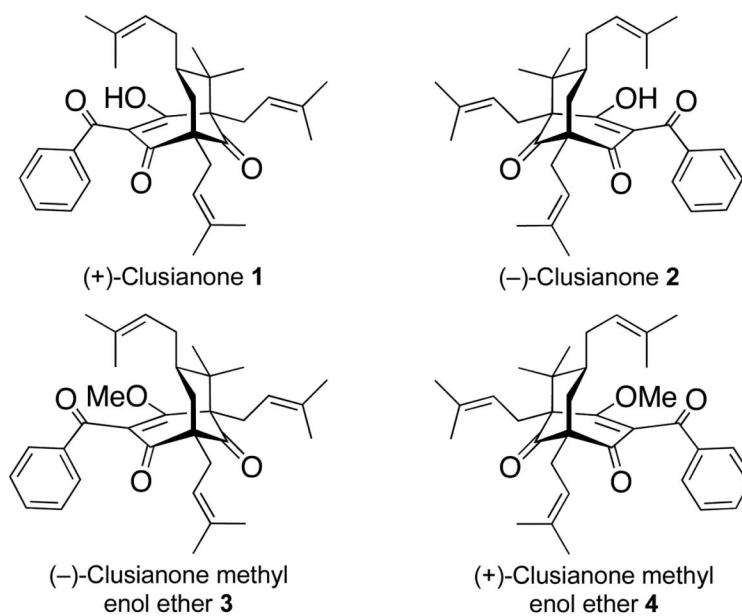
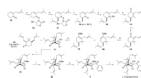**Figure 1.**
The clusianones and their methyl enol ethers.
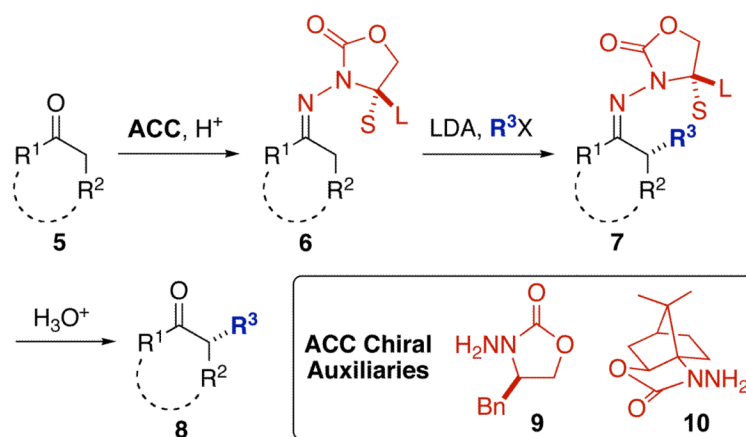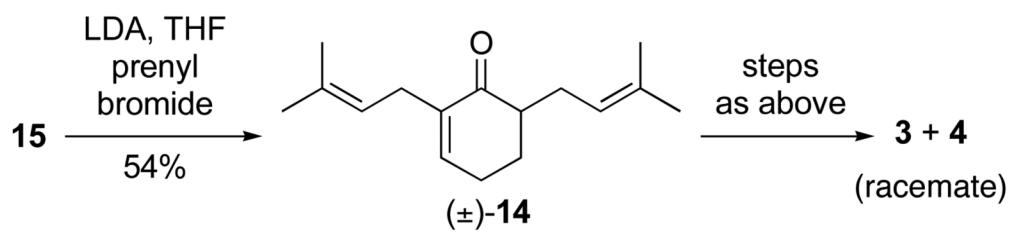
Scheme 1.
Asymmetric ACC α-alkylation of ketones.
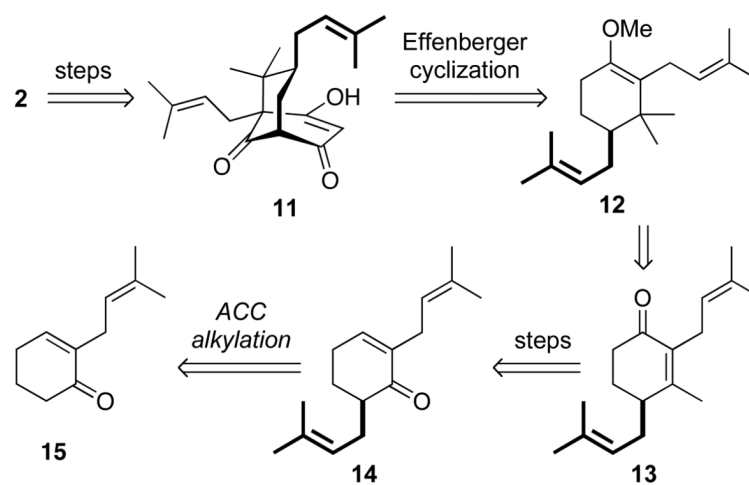
Scheme 2.
Retrosynthetic analysis of **2**.

**Scheme 3.**
Synthesis of (−)-Clusianone (**2**). a) **9**, p-TsOH·H_2O, CH_2Cl_2, reflux, 95%; b) LDA, THF, −78 °C, then prenyl bromide, to 0 °C, 90%; c) p-TsOH·H_2O, acetone, H_2O, 80%; d) MeMgBr, Et_2O, −78 °C to rt, 88%; e) PCC, 3 Å MS, CH_2Cl_2, 67%; f) MeMgBr, CuBr·SMe_2, TMSCl, HMPA, then 10% HCl, 88%; g) t-BuOK, DMSO, then Me_2SO_4, 63%; h) malonyl dichloride, Et_2O, −20 °C, then KOH, $BnEt_3NCl$, H_2O, 35%; i) $(MeO)_3CH$, p-TsOH·H_2O, MeOH, 50 °C, 60%; j) LDA, THF, −78 °C, then prenyl bromide, 90%; k) LTMP, THF, −78 °C, then BzCl, 65%; l) LiOH, dioxane, H_2O, 90 °C, 79%.

Scheme 4.
Preparation of a racemic mixture of **3** and **4**.
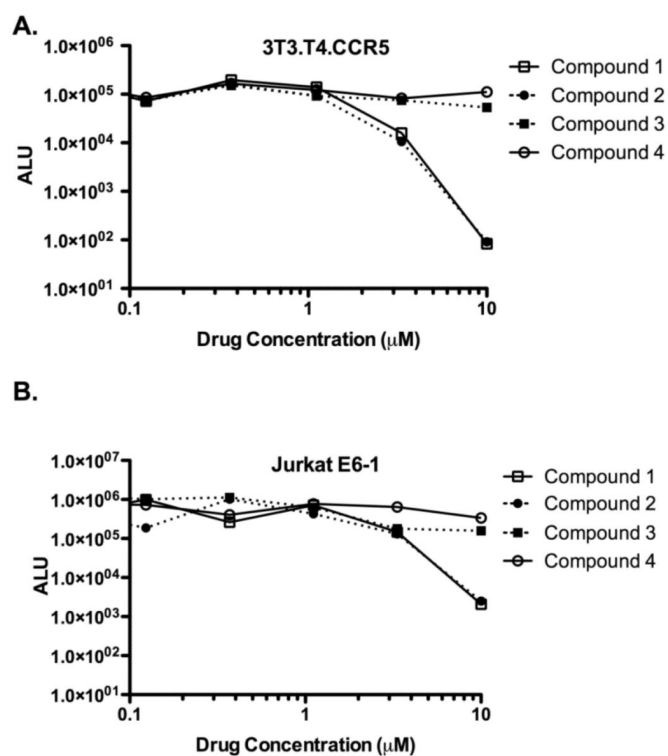
Figure 2.
Compounds **1** and **2** block HIV-1 infection. HIV-1 Env-pseudoviruses were incubated with 3×10^4 3T3.T4.CCR5 (Figure 2A) or Jurkat E6-1 (Figure 2B) cells at varying concentrations of compounds **1–4**. Two days post-infection luciferase activity was measured. Cells with no virus had luciferase values less than 100 arbitrary light units (ALU). Cells with no drug and 1% DMSO had ALU values similar to the values obtained at the 0.005 µM concentration of the drug.