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New hybrids between triterpenoid acids and nucleoside HIV-RT inhibitors

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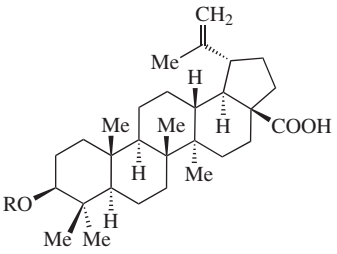
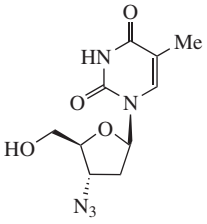
One-pot synthesis of ester-linked conjugates of betulinic, ursolic or oleanolic acids with anti-HIV drugs AZT, derivatives of AZT and 3TC was performed, the resulting conjugates having shown high anti-HIV activities.

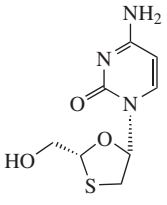
Betulinic acid **1**, ursolic acid and oleanolic acid are naturally occurring pentacyclic triterpenoids whose derivatives possess anticancer, antibacterial, anti-inflammatory, anti-mycosis and anti-HIV activities.[1,2] To improve the potency of acid **1** toward HIV virus, its 3-*O*-esters **2** and **3** were prepared, which showed promising anti-HIV activities.[3,4] On the other hand, certain hybrid-type combinations of HIV drugs such as AZT **4** and d4T, can generate much more effective treatments than single drugs.[5–12] However, data on AZT hybrid with betulin/betulinic acid[11,12] are scarce.



1 R = H (betulinic acid)
2 R = 3,3-dimethylsuccinyl (Bevirimat)
3 R = succinyl



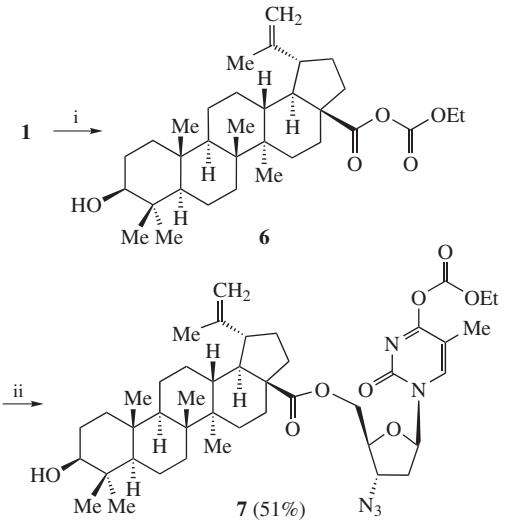
4 (AZT)



5 (3TC)

Here, we report an original synthesis of hybrid compounds of triterpenoids with nucleoside AZT **4**, its derivatives and compound 3TC **5**. The conjugation was performed by the formation of ester bond in a one-pot synthetic procedure.

Attempts to create ester linkage between reactants **1** and **4** using DCC reagent in the presence of DMAP (Steglich reaction), triphenylphosphine/DEAD (Mitsunobu reaction), or EDC/HOBt were unsuccessful, apparently, due to the great steric hindrance. Luckily, when the carboxylic group in compound **1** was first derivatized with ethyl chloroformate, the obtained acyl carbonate intermediate **6** readily reacted with AZT in the presence of Et_3N and DMAP at room temperature to give the desired hybrid



Scheme 1 *Reagents and conditions*: i, 2.0 equiv. EtOC(O)Cl, 1.0 equiv. Et_3N, CH_2Cl_2, 0 °C, 1 h; ii, 1.0 equiv. AZT **4**, 1.0 equiv. Et_3N, 0.5 equiv. DMAP, CH_2Cl_2, room temperature, 12 h.

product **7** in 51% yield (Scheme 1). This protocol was successfully applied to the synthesis of other hybrid compounds[†] (Schemes 2–4). Notably, during the incorporation of carbonate ester group into the AZT moiety, the hydroxyl group at the C-3 position of betulinic acid remained free despite excess of ethyl chloroformate was used. This could be owing to hindrant steric environment around the C-3 position in the betulinic acid residue.

The structure of product **7** was confirmed by NMR and MS spectra. The NMR signal of proton at the C-3 position was similar to that of betulinic acid, which confirmed the free hydroxyl group

[†] *General one-pot procedure for the preparation of hybrid compounds*. A solution of EtOC(O)Cl (0.44 mmol) and Et_3N (0.22 mmol) in CH_2Cl_2 (1 ml) was added dropwise to an ice-cooled solution of the corresponding terpenoid (0.22 mmol) in anhydrous CH_2Cl_2 (10 ml). The mixture was stirred at 0 °C for 1 h to obtain the intermediate activated carbonate. Afterwards, the reaction mixture was added dropwise to a solution of Et_3N (0.22 mmol), corresponding drug (0.22 mmol) and DMAP (0.1 mmol). The mixture was stirred at 0 °C for 1 h, then at room temperature for 12 h. After removal of the solvent, the crude residue was extracted with CH_2Cl_2 (3×20 ml). The combined extracts were washed with brine (3×10 ml) and dried with $MgSO_4$. The solvent was removed under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (CH_2Cl_2/MeOH) to obtain white crystals.

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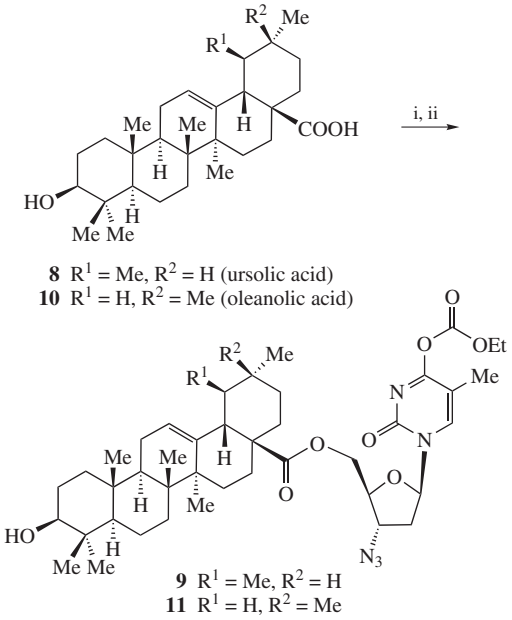


Scheme 2 *Reagents and conditions*: i, 2.0 equiv. EtOC(O)Cl, 1.0 equiv. Et_3N, CH_2Cl_2, 0 °C, 1 h; ii, 1.0 equiv. AZT **4**, 1.0 equiv. Et_3N, 0.5 equiv. DMAP, CH_2Cl_2, room temperature, 12 h.

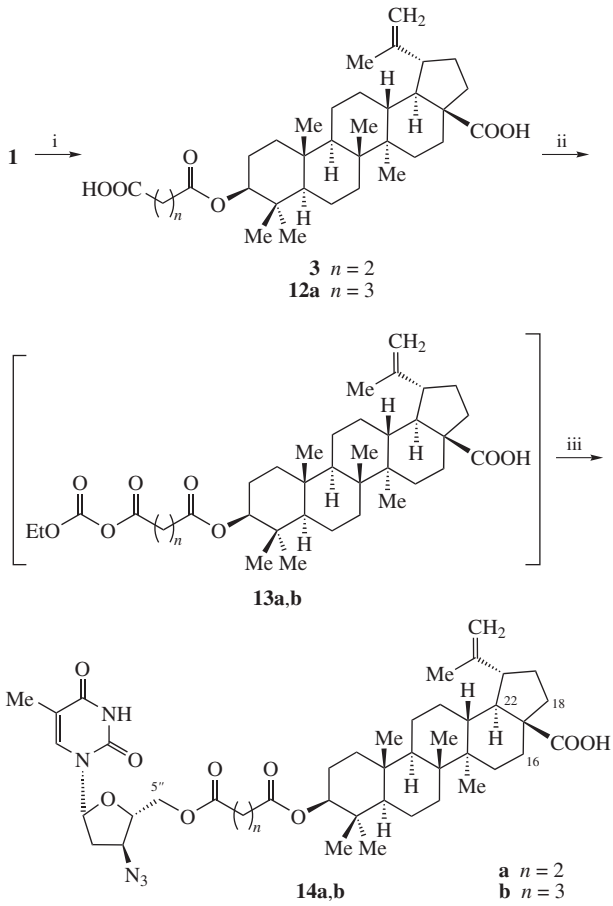
Compound **7**. ^{1}H NMR (500 MHz, $CDCl_3$) (signals typical of terpenoid structure were omitted for clarity) δ: 7.32 (s, 1H, H-6′), 6.10 (t, 1H, H-1″, *J* 6.5 Hz), 4.61 (s, 1H, H-29a), 4.48 (s, 1H, H-29b), 4.32 (dd, 1H, H-5″a, *J* 3.9 Hz), 4.26 (dd, 1H, H-5″b, *J* 3.9 Hz), 4.19–4.12 (m, 3H, 2H-2‴, H-3″), 3.98 (m, 1H, H-4″), 3.05 (t, 1H, H-3, *J* 8 Hz), 2.89 (m, 1H, H-19), 2.39–2.34 (m, 1H, H-2″a), 2.30–2.25 (m, 1H, H-2″b), 2.11 (ddd, 2H, H-15a, H-16a, *J* 2.0, 12.0 and 15.0 Hz), 1.82–1.84 (m, 3H, H-12a, H-22a), 1.81 (s, 3H, H-7′), 1.59 (s, 3H, H-30), 1.56–1.58 (m, 3H, H-1a, H-2a, H-21a), 1.47–1.50 (m, 4H, H-1a, H-2a, H-15b, H-16b), 1.38–1.41 (m, 4H, H-6, H-13a, H-21a, H-22b), 1.25–1.32 (m, 4H, H-7, H-11), 1.22 (t, 3H, CH_2*Me*, *J* 7.0 Hz), 1.15–1.18 (2H, H-9, H-21a), 0.91 (d, 1H, H-5, *J* 11.5 Hz), 0.88 (s, 3H, H-27), 0.86 (s, 3H, H-23), 0.84 (s, 3H, H-26), 0.71 (s, 3H, H-24), 0.64 (s, 3H, H-25), 0.56–0.58 (m, 1H, H-1a). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 179.1 (C-28), 164.2 (C-4′), 154.6 (C-2′), 150.6 (C-20), 150.5 (C-1‴), 135.4 (C-6′), 111.2 (C-5′), 109.3 (C-29), 84.8 (C-1″), 81.6 (C-4″), 78.7 (C-3), 66.1 (C-2‴), 64.7 (C-5″), 59.9 (C-3″), 56.1 (C-17), 55.3 (C-5), 50.5 (C-9), 49.2 (C-18), 46.9 (C-19), 42.3 (C-14), 40.6 (C-8), 38.7 (C-4), 38.2 (C-1), 37.5 (C-13), 37.1 (C-10, C-22), 37.02 (C-2″), 34.3 (C-7), 32.2 (C-16), 30.5 (C-21), 29.6 (C-15), 27.7 (C-23), 26.9 (C-2), 25.5 (C-12), 20.8 (C-11), 19.1 (C-30), 18.2 (C-6), 15.9 (C-25), 15.8 (C-26), 15.2 (C-24), 14.5 (C-27), 13.9 (C-3‴), 12.1 (C-7′). HRMS, *m*/*z*: 778.4753 $[M + H]^+$ (calc. for $C_{43}H_{64}N_5O_8$, *m*/*z*: 778.4749).

Compound **9**. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.25 (s, 1H, H-6′), 6.09 (t, 1H, H-1″, *J* 6.0 Hz), 5.11 (t, 1H, H-12, *J* 3 Hz), 4.30 (dd, 1H, H-5″a, *J* 3.0 Hz), 4.25 (dd, 1H, H-5″b, *J* 3.0 Hz), 4.16 (q, 2H, C*H*$_2$Me), 4.13 (d, 1H, H-3″, *J* 7 Hz), 3.94 (m, 1H, H-4″), 3.06 (t, 2H, H-2″b, H-3, *J* 8.0 Hz), 2.31–2.36 (m, 1H, H-2″a), 2.23–2.29 (m, 1H, H-2″b), 2.07 (d, 1H, H-18, *J* 11.5 Hz), 2.05 (dd, 1H, H_b-22, *J* 13.0 and 4.0 Hz), 1.87 (dd, 2H, H-11, *J* 4.5 and 14 Hz), 1.80 (s, 3H, Me, AZT), 1.74 (dd, 1H, H-19, *J* 4.0 and 13.0 Hz), 1.59 (dd, 1H, H-18, *J* 2.5 and 10 Hz), 1.57 (s, 1H, H-9), 1.54–1.55 (m, 2H, H-1), 1.53–1.54 (m, 1H, H-22b), 1.52–1.53 (m, 2H, H-16), 1.51–1.52 (m, 1H, H-20), 1.46–1.47 (m, 1H, H-6a), 1.41–1.42 (m, 2H, H-2), 1.29–1.30 (m, 2H, H-21), 1.27–1.28 (m, 2H, H-7), 1.25 (s, 3H, Me-23), 0.98 (s, 3H, Me-24), 0.77 (s, 3H, Me-25), 1.08 (s, 3H, Me-26), 1.14 (s, 3H, Me-27), 0.93 (d, 3H, Me-29, *J* 6.5 Hz), 0.91 (d, 3H, Me-30, *J* 5.9 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 180.4 (C-28), 164.1 (C-4′), 154.5 (C-1‴), 150.5 (C-2′), 137.9 (C-6′), 135.3 (C-13), 125.3 (C-12), 111.0 (C-5′), 84.6 (C-1″), 81.4 (C-3″), 78.6 (C-3), 64.6 (C-5″), 59.7 (C-4″), 55.0 (C-5), 52.6 (C-18), 47.6 (C-9), 47.3 (C-17), 39.2 (C-1), 38.8 (C-20), 38.7 (C-19), 38.4 (C-4), 37.3 (C-10), 36.7 (C-22), 32.8 (C-21), 30.4 (C-7), 27.5 (C-2), 26.5 (C-27), 23.9 (C-15), 23.2 (C-11), 23.0 (C-23), 20.8 (C-26), 18.1 (C-30), 16.7 (C-16), 16.6 (C-25), 15.3 (C-29), 15.1 (C-24), 13.8 (C-4‴), 12.0 (C-7′).

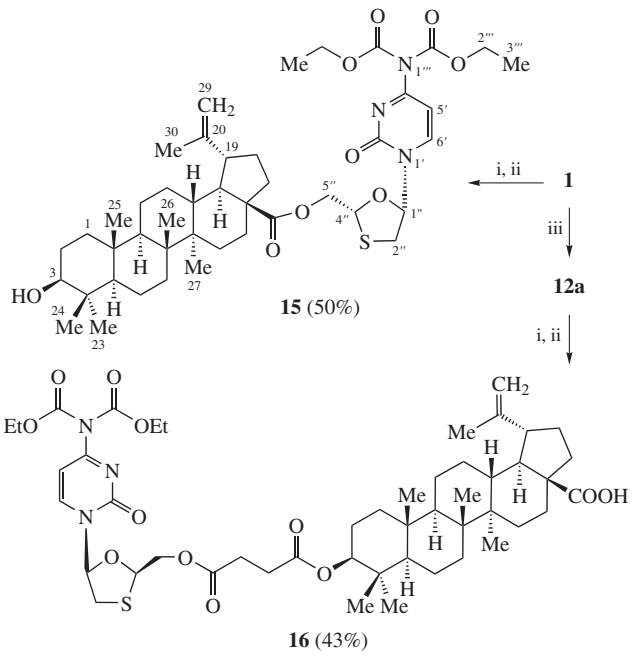
For characteristics of compounds **3**, **6**, **11**, **12a**, **14a**,**b**, **15** and **16**, see Online Supplementary Materials.

at C-3 in the product. The usage of ethyl chloroformate offered an advantage in final product separation and purification, since the only by-products of the process were ethanol and carbon dioxide. This protocol was applied to the synthesis of hybrid compounds between AZT and other pentacyclic triterpenoids (Scheme 2), namely ursolic acid **8** and oleanolic acid **10**, hybrid compounds **9** and **11** having been obtained in 48% and 45% yields, respectively.

Two conjugates, **14a** and **14b**, between AZT and 3-*O*-succinyl (**3**) and 3-*O*-glutaryl (**12a**) betulinic acids were synthesized by straightforward reaction between betulinic acid and succinic (glutaric) anhydrides in dichloromethane in the presence of pyridine and DMAP at reflux for 12 h (Scheme 3).[11(a)] In compounds **3** and **12a**, the remote carboxylic groups at the C-3 substituent were not hindered, so ethyl chloroformate attack occurred selectively at this position to give first the intermediates **13a**,**b**. Condensation of these two intermediates with AZT afforded then the hybrid products **14a**,**b** in 50% and 53% yields, respectively. Their structures were confirmed by extensive NMR studies. HMBC spectra of **14a**,**b** showed interactions between protons at the C-16, C-18 and C-22 positions in betulinic acid moiety with the free carboxylic group at the C-28 position. On the other hand, these HMBC spectra exhibited interactions between two protons at the C-5″ position in the AZT moiety with the ester carbonyl groups, which confirmed the formation of the ester linkage between AZT and the terminal carboxylic group in the side chain. Due to the more facile esterification, equimolar amount of ethyl chloroformate was used instead of excess amount, that led to products **14a**,**b** which did not contain carbonate ester group in the AZT moiety.



Scheme 3 *Reagents and conditions*: i, 1.0 equiv. succinic or glutaric anhydride, 1.0 equiv. pyridine, 0.5 equiv. DMAP, CH_2Cl_2, reflux, 12 h; ii, 2.0 equiv. EtOC(O)Cl, 1.0 equiv. Et_3N, CH_2Cl_2, 0 °C, 1 h; iii, 1.0 equiv. AZT **4**, 1.0 equiv. Et_3N, 0.5 equiv. DMAP, CH_2Cl_2, room temperature, 12 h.



Scheme 4 *Reagents and conditions*: i, 2.0 equiv. EtOC(O)Cl, 1.0 equiv. Et_3N, CH_2Cl_2, 0 °C, 1 h; ii, 1.0 equiv. 3TC **5**, 1.0 equiv. Et_3N, 0.5 equiv. DMAP, CH_2Cl_2, room temperature, 12 h; iii, 1.0 equiv. succinic anhydride, pyridine.

We also synthesized conjugates of betulinic acid and 3-*O*-succinyl derivatives with a drug 3TC **5** (Scheme 4). When applying the same protocol, betulinic acid was conjugated directly with 3TC to give hybrid product **15** in 50% yield. Similarly, condensation of 3TC **5** and 3-*O*-succinyl betulinic acid provided hybrid product **16** in 43% yield. The formation of bis-carbamate at 3TC moiety was observed in these conjugates. Since the reaction yield was about 50%, the excess ethyl chloroformate was enough to react twice with the hybrid products to form the bis-carbamate moiety.

The anti-HIV activities of synthesized hybrid compounds are summarized in Table 1. All conjugates of betulinic, oleanolic and ursolic acids with AZT and its derivatives showed potent activities with EC_{50} values of 0.017 to 0.01 μM. These conjugates also had low toxicity toward MT-4 cell, with SI values above 1400. The conjugates with 3TC **5** did not exhibit high activities and cell-compatibility. We contributed part of this result to the extensive carbamate formation of the 3TC moiety, however, detailed study on this effect is underway.

Table 1 Anti-HIV data against HIV-1_{IIIB} infected MT-4 cells.[a]

Compounds	EC_{50}/μM[b]	CC_{50}/μM[c]	SI[d]
7	0.017	35	2000
9	—	—	—
11	0.010	35	3500
14a	0.015	>50	>3000
14b	0.012	17	1400
15	5	>50	>10
AZT	0.047	>32	>680
3TC	0.330	>100	>300

[a] All anti-HIV data were collected at Tibotec BVBA, Belgium. [b] Concentration that inhibits HIV-1_{IIIB} replication by 50%. [c] Concentration that inhibits mock-infected MT-4 cell growth by 50%. [d] Selectivity index SI = CC_{50}/EC_{50}.

In conclusion, an effective, short synthesis of the conjugates of anti-HIV active triterpenoids with AZT and its derivatives or with 3TC is presented. This direct synthesis provided a scalable procedure for preparing anti-HIV hybrid conjugates with promising activities. All obtained conjugates of triterpenoids with AZT and its derivatives exhibited very good anti-HIV activity. The refinement of conjugate structures and exploration of other combinations are under active investigation.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.03.004.

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