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Nucleoside homodimerisation by cross metathesis

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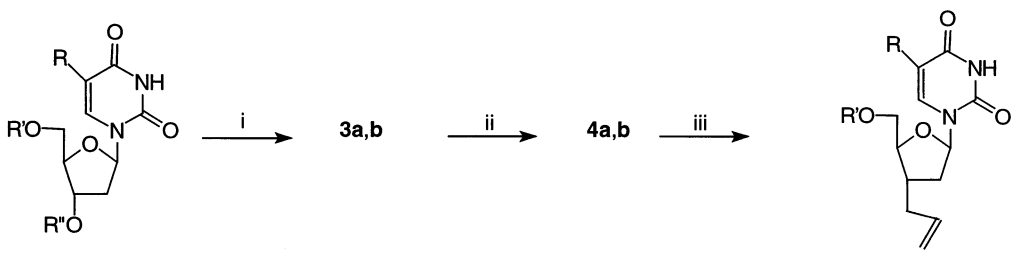
Abstract—The cross metathesis of 3′-allylic analogues of thymidine, 2′-deoxyuridine and 2′-deoxycytidine is used to obtain nucleoside dimers, in which an unsaturated hydrocarbon chain links the 3′ positions of the sugar moieties. The biological activity on HIV infected cells was also evaluated.

Metathesis is receiving a renewal of interest since the discovery of new and efficient catalysts such as the ruthenium carbene complexes recently introduced by Grubbs and co-workers.[1] It can be used for many synthetic applications: ring opening polymerization,[1,2] cross-metathesis between identical or different terminal olefins[3–5] and ring-closing metathesis[6] which is the most used application of this technique. Thanks to this progress, we have planned the synthesis of nucleoside dimers, analogues of dinucleotides. It is now well known that nucleoside and oligonucleotide analogues[7] possess therapeutic activities in particular against retro-viruses. With the purpose of getting new active molecules, we describe herein the synthesis of dinucleosides linked by an olefinic chain between the positions 3′ of their glycosidic moiety, using a cross metathesis reaction. Such a hydrocarbon chain should enhance the lipophilic character of the oligonucleotide analogues and help penetration of these compounds into cells. To the best of our knowledge, metathesis has not been applied to nucleosides yet.

For the choice of a catalyst, we decided to use a ruthenium carbene complex developed by Grubbs and co-workers,[1] benzylidenebis(tricyclohexylphosphine)dichloro-ruthenium(IV) (**1**), for its high reactivity, stability and remarkable functional group tolerance.



2a: R = CH_3, R' = H, R" = H
2b: R = H, R' = H, R" = H

3a: R = CH_3, R' = TBDPS, R" = H
3b: R = H, R' = TBDPS, R" = H

4a: R = CH_3, R' = TBDPS, R" = C(S)OΦ
4b: R = H, R' = TBDPS, R" = C(S)OΦ

5a: R = CH_3, R' = TBDPS
5b: R = H, R' = TBDPS

Scheme 1. (i) TBDPSCl (1.2 equiv.), DMAP (0.02 equiv.), dry pyridine; (ii) PhOC(S)Cl (1.1 equiv.), DMAP (2 equiv.), dry acetonitrile; (iii) $Bu_3SnCH_2CH{=}CH_2$ (4 equiv.), dry toluene.

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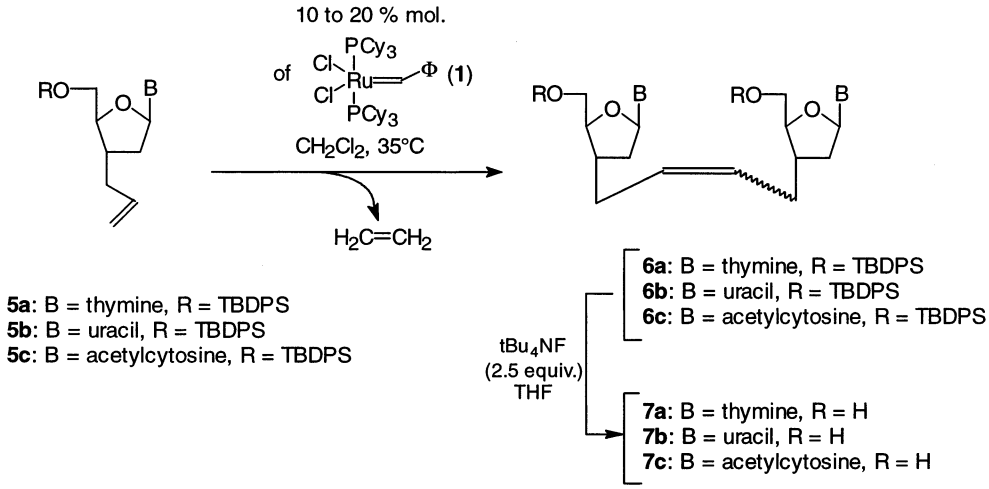

Scheme 2.

The allylic nucleosides (**5a,b**) (Scheme 1) are obtained in three steps starting from the corresponding 2′-deoxynucleosides. The first one is the selective protection of the 5′-hydroxy group of 2′-deoxynucleoside (**2a,b**), using *t*-butyldiphenylsilyl chloride (1.1 equiv.) in dry pyridine in presence of DMAP (0.02 equiv.) giving the 5′-selectively protected product (**3a,b**) in high yields (90–95%). Next, the 3′-hydroxy moiety is changed to the corresponding phenylthionocarbonate (**4a,b**) by reaction with phenylchlorothionocarbonate (1.1 equiv.) in dry acetonitrile with DMAP (2 equiv.).[8] The 3′-allyl-5′-*t*-butyldiphenylsilyl-2′,3′-dideoxy nucleoside[9] (**5a,b**) is obtained after a free radical reaction with allyltributyltin (4 equiv.) in dry toluene with AIBN as radical initiator.

This strategy is not efficient to prepare the allylcytidine analogue:[10] the yields of the two last steps are very low (less than 10%), even if the silyl-ether protection system is modified by a selective benzoylation on N-4 and O-5′ position. Finally for the obtention of the cytidine derivative, we decided to use the allyluridine (**5b**) already synthesized and change the C-4 carbonyl group into an amino moiety in two steps:[11] firstly, the carbonyl moiety is converted into a thiocarbonyl with Lawesson's reagent (0.75 equiv.) in dry 1,2-dichloroethane (90%); secondly, this thiouridine is reacted with methanolic ammonia to give the expected 3′-allyl-5′-*t*-butyldiphenylsilyl-2′,3′-dideoxycytidine (50%).

The second step consists of the self cross metathesis reaction (Scheme 2).† Grubbs's ruthenium catalyst (**1**) exhibits a significant activity in the presence of a large variety of functional groups. Here, nucleosides contain pyrimidine bases with amido groups, the hydroxyl moiety of the sugar parts is protected by a silyl-ether. The metathesis is effected in dry dichloromethane at reflux with 10–20% mol. catalyst (**1**) for 6 h (a longer reaction time does not improve the yield). The yield is about 45% with both thymidine and the 2′-deoxyuridine dimer. The metathesis reaction has also been attempted using the 5′-hydroxyl unprotected compound in the case of the thymidine analogue: the expected product (**7a**) is obtained with a lower yield (15%).

The case of the allylcytidine metathesis is not so straightforward: without protecting the amino group, metathesis does not occur with any significant conversion. A convenient acetylation of the amino moiety[12] (**5c**) is led under microwave irradiation in a domestic oven, with acetic anhydride (2 equiv.) in DMF, in 2 min. After acetylation, metathesis is realized in 20 h and the (*Z*/*E*) isomers have been partially isolated by preparative TLC, in 15% global yield.

In each case, the dimeric final products are obtained after deprotection of the 5′-hydroxyl group with *t*-butylammonium fluoride (2.5 equiv.) in THF in about 70% yield.

The separation of the *Z* and *E* isomers of the thymidine and uridine dimers is not as easy as in the case of the cytidine dimer: the separation of the isomeric mixture can be carried out only after deprotection of the 5′-hydroxyl group, by HPLC on a reverse phase Prep-Nova Pack C18 column (WATERS). The *Z*/*E* ratio so determined is about 55:45 for the uridine and thymidine products. In the NMR spectra, ^{1}H chemical shifts do not allow the identification of the *Z* and *E* isomers but ^{13}C chemical shifts of the carbon atom close to the double bond (C-α) are different for the *Z* and *E* isomers.[13] The upper value ($\delta_{\alpha}=35.85$ ppm) is assigned to the *E* isomer (eluting last) and the lower one ($\delta_{\alpha}=30.33$

† Typical procedure for metathesis reaction: 211 mg (0.43 mmol) of **5b** dissolved in 3.5 mL of dry dichloromethane are introduced in a round bottom flask, under argon atmosphere, 49 mg (0.06 mmol) of **1** dissolved in 1 mL of dry dichloromethane are added slowly. Under stirring, the purple reaction mixture is heated to 35°C for 6 h. The solvent of resulting dark solution is removed under reduced pressure. The residue is purified by preparative TLC ($CHCl_3$/EtOH 95/5) to give 93 mg of **6b** (45%) as a foam.

ppm) to the *Z* isomer in the case of 2′-deoxyuridine dimer (**7b**).‡

Trying to explain the moderate yields obtained for metathesis dimerisation of nucleosides, compared to those obtained for example with sugar derivatives, we can notice that metathesis with N containing products affords lower yields.[6] This could be due to an inhibition of the catalyst by nitrogen atoms[14] that are present in pyrimidines and especially in cytosine. However, trying the metathesis reaction with an excess of catalyst does not improve the yield.

The biological activities of thymidine (**7a**, mix of both isomers) and uridine (**7b**, *E* isomer) dimers were evaluated on CEM-SS cells infected by HIV-1 LAI virus and on MT4 cells infected by HIV-1 IIIB according to standardised protocols.[15] These compounds do not show any activity against these viruses and are not cytotoxic ($IC_{50}>10^{-1}$ mg/mL).

In summary, this paper presents for the first time an application of cross coupling olefin metathesis for the synthesis of dimeric nucleoside analogues. Others analogues are currently under investigation in our laboratory.

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‡ Structure of all products have been checked by NMR (Bruker DPX-400, 400 MHz for ^{1}H, 100 MHz for ^{13}C) and mass spectrometry. Selected NMR values for final compound of dimeric uridine (**7b**) in CD_3OD: E isomer: ^{1}H: 8.11 (d, 2H, $J=8.1$ Hz, H-5), 6.01 (dd, 2H, $J=6.3$, 3.6 Hz, H-1′), 5.65 (d, 2H, $J=8.1$ Hz, H-6), 5.53 (m, 2H, CH=CH), 3.87 (dd, 2H, $J=12.2$, 2.3 Hz, H-5′a), 3.74 (m, 2H, H-4′), 3.68 (dd, 2H, $J=12.2$, 3.6 Hz, H-5′b), 2.30∼2.05 (m, 10 H, H-2′, H-2″, H-3′, $C\underline{H}_2$-CH=). ^{13}C: 166.50 (C-4), 152.28 (C-2), 142.73 (C-5), 130.97 (CH=CH), 101.90 (C-6), 87.95 (C-4′), 86.83 (C-1′), 62.46 (C-5′), 39.90 (C-2′), 38.21 (C-3′), 35.85 ($C\underline{H}_2$-CH=). *Z* isomer: ^{1}H: 8.12 (d, 2H, $J=8.1$ Hz, H-5), 6.04 (dd, 2H, $J=6.4$, 3.6 Hz; H-1′), 5.65 (d, 2H, $J=8.1$ Hz, H-6), 5.53 (m, 2H, CH=CH), 3.89 (dd, 2H, $J=12.2$, 2.4 Hz, H-5′a), 3.77 (m, 2H, H-4′), 3.71 (dd, 2H, $J=12.2$, 3.6 Hz, H-5′b), 2.34∼2.05 (m, 10 H, H-2′, H-2″, H-3′, $C\underline{H}_2$-CH=). ^{13}C: 166.52 (C-4), 152.28 (C-2), 142.72 (C-5), 129.75 (CH=CH), 101.92 (C-6), 88.01 (C-4′), 86.78 (C-1′), 62.46 (C-5′), 39.93 (C-2′), 38.44 (C-3′), 30.33 ($C\underline{H}_2$-CH=).

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