Tetrahedron: *Asymmetry* 19 (2008) 1204–1209

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: http://www.elsevier.com/locate/tetasy

Diastereoselective synthesis of a collection of new homonucleoside mimetics containing pyrrolo[1,2-*b*]isoxazoline and pyrrolidine rings

Vanni Mannucci [a], Franca M. Cordero [a], Anna Piperno [b], Giovanni Romeo [b], Alberto Brandi [a,*]

[a] *Department of Organic Chemistry 'Ugo Schiff', Laboratory of Design, Synthesis and Study of Biologically Active Heterocycles-HeteroBioLab, University of Firenze, via della Lastruccia 13, I-50019 Sesto Fiorentino (FI), Italy*
[b] *Dipartimento Farmaco-Chimico, Viale SS. Annunziata, Università di Messina, Italy*

ARTICLE INFO

Article history:
Received 20 March 2008
Accepted 24 April 2008

ABSTRACT

The 1,3-dipolar cycloaddition of enantiopure cyclic hydroxylated nitrones and allyl nucleobases has been exploited for the preparation of a novel class of homonucleoside mimetics, where the furanose ring is replaced by a pyrrolo[1,2-*b*]isoxazolidine system. The nitrones were easily prepared starting from L-malic and L-tartaric acids and gave cycloadducts in a diastereoselective manner, which were deprotected to give good yields of the homo-N,O-nucleoside mimetics. The reduction of the isoxazolidine ring, a 1,3-aminoalcohol equivalent, allows easy access to other new pyrrolidine nucleoside mimetics.

1. Introduction

Over the last few years, the synthesis of nucleoside analogues with the sugar moiety and/or the heterocyclic base modified have received a great deal of attention for their biological action as antiviral and/or anticancer agents.[1] In particular, isoxazolidine homo-N-nucleosides, synthesized by the cycloadditions of acyclic nitrones and allyl nucleobases in a diastereoselective[2] or enantioselective[3] way, have emerged as an important class of dideoxynucleoside analogues.[4,5]

The 1,3-dipolar cycloaddition of enantiomerically pure pyrroline nitrones is a powerful synthetic tool that allows up to three new stereogenic centers to be assembled in a stereospecific manner in a single step. Moreover, the resulting bicyclic isoxazolidines have been extensively used as 1,3-aminoalcohol equivalents, by taking advantage of the labile nature of the N–O bond under mildly reducing conditions, to obtain a wide variety of natural products, such as alkaloids and related compounds.[6]

Continuing our interest in the synthesis of mimetics of nucleosides,[7] we have focused our attention on the synthesis of a new class of homo-N-nucleoside analogues in which the sugar unit is replaced by a pyrrolo[1,2-*b*]isoxazoline ring and separated by a methylene bridge from the nucleobase. *N*-Allyl pyrimidine nucleobases[8] were chosen as convenient starting materials, together with five-membered enantiopure cyclic nitrones synthesized from L-tartaric acid and L-malic acid.[9]

Once these analogues has been obtained, our interest was then directed to the exploitation of the reactivity of the N–O bond of the isoxazolidine ring in order to synthesize another novel class of nucleoside mimetics characterized by a higher flexibility, which is likely to be beneficial for their interaction with receptors. Recently, it has been demonstrated that the binding sites of many enzymes are more flexible than previously thought and, as a direct consequence, more flexible inhibitors could show better features.[10]

2. Results and discussion

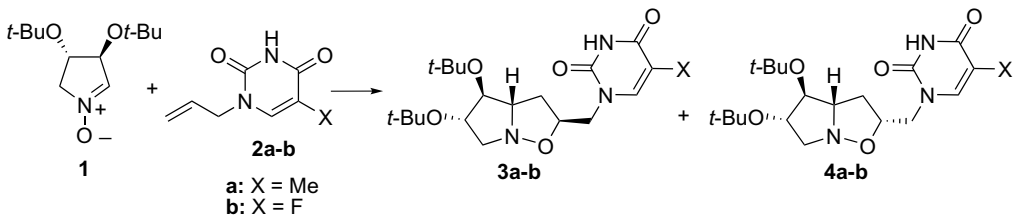
A study of the optimal cycloaddition reaction conditions was carried out employing (3*S*,4*S*)-3,4-di-*tert*-butoxy-1-pyrroline N-oxide **1** with *N*-allylthymine **2a** (Scheme 1, Table 1, entries 1–3). The reaction in toluene at 80 °C, under traditional heating in an oil bath, proceeded smoothly although slowly, requiring 72 h for quantitative conversion and recovery of cycloadducts in 71% (entry 1).

Switching to microwave irradiation, at a temperature of either 80 or 120 °C, an acceleration of the reaction was observed, proportional to the temperature employed. Unfortunately, microwave heating caused a considerable decrease in the yield, probably due to the simultaneous acceleration of decomposition processes (Table 1, entries 2 and 3).

In every case, the same diastereomeric mixture of adducts **3a** and **4a** was obtained in a roughly 7:1 ratio (Table 1, entries 1–3). This result is further evidence that the role of microwaves is only restricted to the efficiency in the transfer of heating to the reaction mixture.[11] Consequently, we decided to apply traditional heating to the cycloaddition with *N*-allyl 5-fluorouracil **2b**, observing a slight increase in both reactivity and stereoselectivity with respect to **2a**. Diastereomeric pyrroloisoxazolines **3b** and **4b** were obtained in a 10:1 ratio in 66 h (Table 1, entry 4). In all the cases, the major diastereomers were successfully separated by flash chromatography.

* Corresponding author. Tel.: +39 055 4573485; fax: +39 055 4573572.
E-mail address: alberto.brandi@unifi.it (A. Brandi).

0957-4166/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2008.04.028



Scheme 1.

Table 1
Cycloadditions of nitrones **1** and **5** with allyl pyrimidines **2**

	Nitrone	Allylbase	*T* (°C)	Time (h)	3:4/6:7 Ratio	Yield (%)
1	**1**	**2a**	80	72	88:12	71
2	**1**	**2a**	80[a]	5	88:12	52
3	**1**	**2a**	120[b]	2	88:12	50
4	**1**	**2b**	80	66	91:9	76
5	**5**	**2a**	80	72	84:16	97
6	**5**	**2b**	80	56	80:20	95

All the reactions have been carried out in a sealed tube with toluene as solvent.
[a] Heated by microwave irradiation at 150 W.
[b] Heated by microwave irradiation at 200 W.

The scarce reactivity observed for *N*-allyl pyrimidines toward nitrone **1** is not surprising. As a matter of fact, the electron-rich nature of the cyclic nitrone **1** makes the interaction unfavorable with an electron-rich dipolarophile as **2a** and **2b**. Nevertheless, practical yields of the major diastereomerically pure products could be afforded as the diastereoselectivity of the processes was quite good.

By applying the same reaction conditions to the cycloadditions between the (3*S*)-3-*tert*-butoxy-1-pyrroline N-oxide **5** and the allyl pyrimidines **2**, a similar trend was observed. In addition, a much higher reactivity, leading to an almost quantitative formation of the adducts, a good, albeit lower (4:1), diastereoselectivity in favor of diastereomers **6** was observed (Scheme 2, Table 1, entries 5 and 6).

The assignment of *anti*(3-*Ot*-*Bu*)-*exo* and *anti*(3-*Ot*-*Bu*)-*endo* structure to adducts **3**, **6** and **4**, **7**, respectively, was made on the basis of the well-known reactivity of the five-membered cyclic nitrones, as well as on the basis of spectroscopic data. In fact, *syn* attack of the dipolarophile is usually highly hindered by unfavorable steric interactions between the dipolarophile and the alkoxy substituent at C-3, an interaction that is not present in the case of the *anti* approach.[6,12] The steric hindrance, in the absence of secondary orbital interactions, is also responsible for the preference for the *exo* approach instead of the *endo*. A J_{trans} for 3a-H and 4-H coupling is observed for all major isomers **3** (J = 4.3–4.5 Hz), **6** (J = 3.6–3.9 Hz), and for the sole minor adduct that was possible to analyze **7a** (J = 2.8 Hz), attesting the *anti* approach of the reagents. Lacking vicinal couplings with the 2-H proton, only NOE experiments could be used to establish the *exo*/*endo* relationship of the products.

In the cases of compounds **6a** and **7a**, structural determination with the aid of NOESY 2D experiments could be performed, which confirmed the assignment. In fact, for the major isomer **6a**, diagnostic correlation peaks between protons 2-H and 4-H and between the methyl groups of the *t*-Bu and *tert*-BuO with 3a-H were found, which is clearly indicative of a *cis* relationship between protons 2-H and 4-H and also between the *t*-BuO group and 3a-H. At the same time, for the minor diastereomer **7a**, a correlation peak between the *t*-Bu methyl groups and 3a-H was still observed, while the one between 2-H and 4-H was absent, thus confirming the *trans* relationship between these two protons (see Fig. 1).

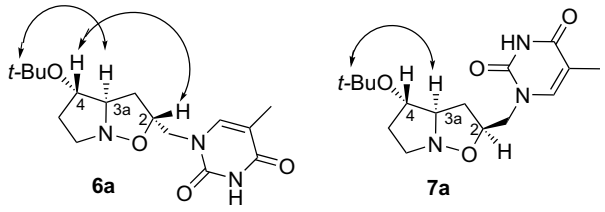
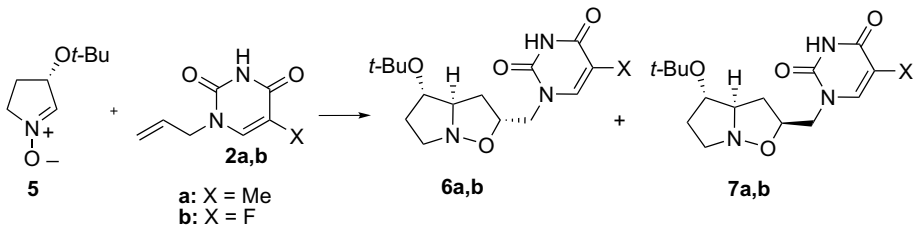


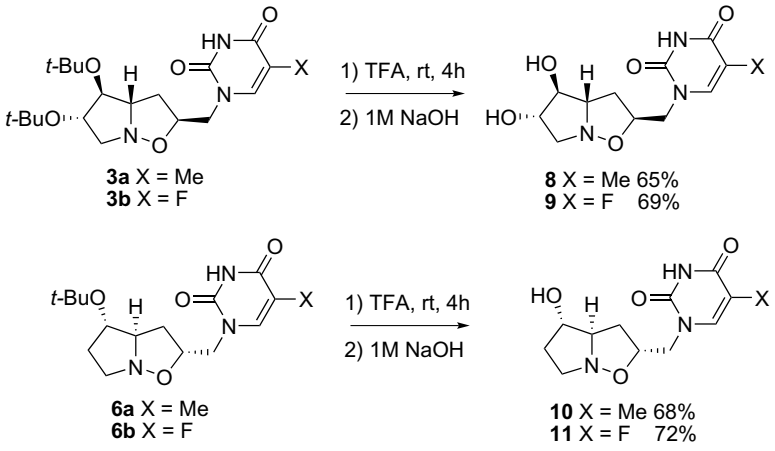
Figure 1. Selected NOESY correlation peaks observed for **6a** and **7a**.

The partial loss of diastereoselectivity observed with nitrone **5** could be explained by the structural differences between the two nitrones. In the case of **1**, the hindrance for the *endo* approach appears to be higher because of the presence of the *t*-BuO group at C-4 of the pyrrolidine ring, which is absent in the case of nitrone **5**. The observed lower reactivity can also be explained by considering this increased hindrance.

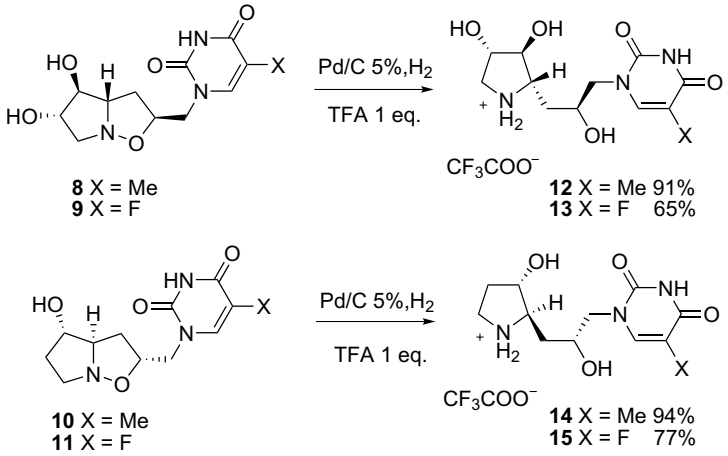
The removal of the protecting groups, by treatment with TFA, afforded the corresponding O-deprotected nucleoside mimetics **8**–**11**, with yields ranging from 65% to 72% (Scheme 3). The unexpected low yield of hydrolysis was due to difficulties experienced during the purification step. In fact, the persistent presence of sodium trifluoroacetate, a by-product of the work-up, was observed in the products even after chromatography. This behavior is probably caused by the solubility of sodium trifluoroacetate in



Scheme 2.



Scheme 3.



Scheme 4.

the eluents[13] used and to a co-solubility effect between substrates **8**–**11** and the salt.[14] Unfortunately, all attempts to find alternative methods of purification failed.[15]

Once we had obtained the pyrrolo[1,2-*b*]isoxazoline type nucleoside mimetics, our attention was focused on the reductive opening of the isoxazolidine ring. Palladium on C (10%) in the presence of trifluoroacetic acid (1 equiv) revealed good efficiency in the hydrogenolysis of the N–O bond (Scheme 4). The compounds obtained, due to their nature and instability, were preferably isolated as the corresponding trifluoroacetate salts. The lower yields of the fluorouracil derivatives **13** and **15** can be easily explained by considering the lability of the double bond of the pyrimidine ring, due to the fluorine substitution.[16]

3. Conclusion

A practical approach to a series of nucleoside mimetics containing a pyrrolo[1,2-*b*]isoxazoline or a pyrrolidine ring has been outlined. The synthesis is based on a diastereoselective cycloaddition reaction between enantiopure cyclic nitrones, derived from L-malic and L-tartaric acid, and allyl nucleobases.

The absolute configuration, derived from the starting chiral pool hydroxy acids, and from the cycloaddition process, confers to the products containing the same nucleobase, a quasienantiomeric relationship that could be of interest in the study of biological activity.

4. Experimental

4.1. General information

Melting points were measured on a Kofler apparatus and are uncorrected. NMR spectra were measured on a Varian Mercury Plus 400 MHz or a Varian Gemini 200 MHz instruments; chemical shifts are in ppm (δ) from TMS as internal standard. IR spectra were recorded using a Perkin–Elmer Spectrum BX (FT-IR system) spectrophotometer; when measured on the neat product, the compound has been applied over a layer of CaF_2. Polarimetric measures were performed with a Jasco DIP-370. Mass spectra were recorded with a Shimadzu GCMS-QP550 instrument by direct inlet and 70 eV; relative percentages are shown in brackets. Exact mass spectra were recorded with a LTQ Orbitrap instrument. Elemental analyses were performed with a Perkin–Elmer 2400 analyzer. R_f values refer to TLC on 0.25 mm silica gel plates.

4.2. Synthesis of pyrrolo[1,2-*b*]isoxazolines 3a, 3b, 6a, and 6b

General procedure: A solution of the corresponding nitrone (1 equiv) and allylnucleobase (1.1 equiv) in 20 mL of toluene in a sealed tube was heated at 80 °C. The solution was then evaporated under reduced pressure and the residue purified by flash chromatography (with pure EtOAc as solvent) to give the respective pure major pyrrolo[1,2-*b*]isoxazoline cycloadducts. The minor isomers

were obtained only in mixture with the major ones and, apart compound **7a**, could not be analyzed spectroscopically.

4.2.1. 1-{[(2*S*,3a*S*,4*S*,5*S*)-4,5-Di-*tert*-butoxyhexahydropyrrolo-[1,2-*b*]isoxazol-2-yl]methyl}-5-methyl pyrimidine-2,4(1*H*,3*H*)-dione 3a

The reaction of nitrone **1** (504 mg, 2.2 mmol) and allylbase **2a** (402 mg, 2.42 mmol) was carried out for 72 h, thus obtaining, after purification by FCC, the pure compound **3a** (540 mg, 62%). R_f = 0.24. White solid; mp 158–159 °C. $[\alpha]_D^{25} = +90$ (*c* 1.1, $CHCl_3$). δ_H (400 MHz, 25 °C, $CDCl_3$) 8.35 (br s, 1 H, NH), 7.16 (br s, 1H, =CH), 4.51 (dddd, 1H, 2-H, *J* = 7.8, 7,2, 6.9, 2.4 Hz), 4.11 (dd, 1H, CH_2N_{base}, *J* = 14.3, 2.4 Hz), 3.89 (ddd, 1H, 5-H, *J* = 6.9, 5.9, 4.5 Hz), 3.77 (t, 1H, 4-H, *J* = 4.5 Hz), 3.55 (dd, 1H, CH_2N_{base}, *J* = 14.3, 7.8 Hz), 3.51–3.43 (m, 1H, 3a-H), 3.47 (dd, 1H, 6-H, *J* = 11.3, 5.9 Hz), 2.87 (dd, 1H, 6-H, *J* = 11.3, 6.9 Hz), 2.38 (ddd, 1H, 3-H, *J* = 12.5, 6.9, 5.2 Hz), 2.14 (ddd, 1H, 3-H, *J* = 12.5, 9.2, 7.2 Hz), 1.89 (d, 3H, CH_3, *J* = 1.2 Hz), 1.18 (s, 9H, *tert*-Bu), 1.16 (s, 9H, *tert*-Bu); δ_C (50 MHz, 25 °C, $CDCl_3$) 164.5, 151.3, 141.8, 110.1, 81.9, 76.6, 74.8, 74.1, 74.0, 70.1, 60.3, 49.7, 37.0, 28.7, 28.4, 12.3. MS (70 eV, EI): *m*/*z* (%) 395 (5), 338 (5), 282 (10), 210 (8), 144 (10), 96 (16), 84 (27), 57 (100). IR ($CDCl_3$): 3383, 3176, 3045, 2977, 1685, 1467, 1366, 1244, 1190 cm^{-1}. Anal. Calcd for $C_{20}H_{33}N_3O_5$: C, 60.74; H, 8.41; N, 10.62. Found: C, 60.97; H, 8.36; N, 10.88.

4.2.2. 1-{[(2*S*,3a*S*,4*S*,5*S*)-4,5-Di-*tert*-butoxyhexahydropyrrolo-[1,2-*b*]isoxazol-2-yl]methyl}-5-fluoropyrimidine-2,4(1*H*,3*H*)-dione 3b

The reaction of nitrone **1** (490 mg, 2.14 mmol) and allylbase **2b** (400 mg, 2.35 mmol) was carried out for 66 h thus obtaining, after purification by FCC, the pure compound **3b** (590 mg, 69%). R_f = 0.13. White solid; mp 181–182 °C. $[\alpha]_D^{25} = +116$ (*c* 0.775, $CHCl_3$). δ_H (400 MHz, 25 °C, $CDCl_3$) 7.49 (d, 1H, =CH, *J* = 5.8 Hz), 4.64–4.46 (m, 1H, 2-H), 4.11 (dd, 1H, CH_2N_{base}, *J* = 14.3, 2.3 Hz), 3.90 (dt, 1H, 5-H, *J* = 6.0, 4.9 Hz), 3.77 (t, 1H, 4-H, *J* = 4.3 Hz), 3.59–3.42 (m, 3H, 3a-H + CH_2N_{base} + 6H), 2.90 (dd, 1H, 6-H, *J* = 11.8, 6.5 Hz), 2.40 (ddd, 1H, 3-H, *J* = 12.6, 7.2, 5.2 Hz), 2.14 (ddd, 1H, 3-H, *J* = 12.6, 8.8, 6.6 Hz), 1.15 (s, 9H, *tert*-Bu), 1.14 (s, 9H, *tert*-Bu); δ_C (50 MHz, 25 °C, $CDCl_3$) 157.3, (d, $J_{CF}^2 = 26.0$ Hz), 149.8, 139.7 (d, $J_{CF}^1 = 236.0$ Hz), 129.9 (d, $J_{CF}^2 = 33.0$ Hz), 81.6, 76.8, 74.6, 73.8, 69.7, 60.2, 49.8, 36.5, 28.5, 28.1 (2C). MS (70 eV, EI): *m*/*z* (%) 399 (25), 342 (20), 286 (50), 270 (15), 214 (32), 144 (50), 100 (26), 84 (39), 57 (100). IR ($CDCl_3$): 3383, 3176, 3076, 2977, 1701, 1664, 1470, 1366, 1242, 1190 cm^{-1}. Anal. Calcd for $C_{19}H_{30}FN_3O_5$: C, 57.13; H, 7.57; N, 10.52. Found: C, 57.18; H, 7.95; N, 10.66.

4.2.3. 1-{[(2*R*,3a*R*,4*S*)-4-*tert*-Butoxyhexahydropyrrolo[1,2-*b*]-isoxazol-2-yl]methyl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione 6a

The reaction of nitrone **5** (471 mg, 3.0 mmol) and allylbase **2a** (548 mg, 3.3 mmol) was carried out for 72 h thus obtaining, after purification by FCC, the pure compound **6a** (786 mg, 81%). R_f = 0.31. White solid; mp 60–61 °C. $[\alpha]_D^{25} = -103$ (*c* 0.325, $CHCl_3$). δ_H (400 MHz, 25 °C, $CDCl_3$) 10.17 (br s, 1H, NH), 7.16 (q, 1H, =CH, *J* = 1.2 Hz), 4.32–4.25 (m, 1H, 2-H), 4.03 (dd, 1H, CH_2N_{base}, *J* = 14.1, 2.6 Hz), 3.82 (dt, 1H, 4-H, *J* = 7.4, 3.9 Hz), 3.50–3.44 (m, 1H, 3a-H), 3.42 (dd, 1H, CH_2N_{base}, *J* = 14.1, 9.1 Hz), 3.29 (A part of ABMX system, 1H, 6-H, *J* = 13.3, 8.7, 7.2 Hz), 3.19 (B part of ABMX system, 1H, 6-H, *J* = 13.3, 7.3, 4.4 Hz), 2.25 (ddd, 1H, 3-H, *J* = 10.4, 7.2, 3.1 Hz), 2.22–2.08 (m, 2H, 3-H + 5-H), 1.83 (d, 3H, CH_3, *J* = 1.2 Hz), 1.62 (m, 1H, 5-H), 1.12 (s, 9H, *tert*-Bu); δ_C (50 MHz, 25 °C, $CDCl_3$) 164.4, 151.0, 141.8, 109.6, 77.8, 74.7, 73.4, 72.6, 54.8, 50.1, 37.1, 33.3, 28.2, 12.1. MS (70 eV, EI): *m*/*z* (%) 323 (21), 266 (48), 210 (18), 140 (29), 128 (65), 96 (55), 84 (38), 57 (100). IR ($CDCl_3$): 3394, 3176, 2977, 1709, 1469, 1365, 1318, 1249, 1216, 1188 cm^{-1}. Anal. Calcd for $C_{16}H_{25}N_3O_4$: C, 59.42; H, 7.79; N, 12.99. Found: C, 59.09; H, 8.18; N, 12.80.

4.2.4. 1-{[(2*S*,3a*R*,4*S*)-4-*tert*-Butoxyhexahydropyrrolo[1,2-*b*]-isoxazol-2-yl]methyl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione 7a

δ_H (400 MHz, 25 °C, $CDCl_3$): 9.25 (br s, 1H, NH), 7.22 (q, 1H, =CH, *J* = 1.1 Hz,), 4.29 (qd, 1H, 2-H, *J* = 7.0, 2.4 Hz), 4.07 (dd, 1H, CH_2N_{base}, *J* = 14.2, 2.5 Hz), 4.10–4.04 (m, 1H, 4-H), 3.65 (ddd, 1H, 3a-H, *J* = 9.3, 7.2, 2.8 Hz), 3.52 (dd, 1H, CH_2N_{base}, *J* = 14.2, 8.4 Hz), 3.21 (ddd, 1H, 6-H, *J* = 12.6, 7.3, 5.1 Hz), 3.02 (ddd, 1H, 6-H, *J* = 12.8, 8.8, 6.9 Hz), 2.64 (ddd, 1H, 3-H, *J* = 12.4, 7.1, 2.8 Hz), 1.95–1.80 (m, 3H, 3-H + 5-H + 5-H), 1.88 (d, 3H, CH_3, *J* = 1.2 Hz), 1.16 (s, 9H, *t*-Bu). δ_C (50 MHz, 25 °C, $CDCl_3$): 164.6, 151.2, 142.1, 109.8, 76.4, 73.9, 71.6, 68.0, 53.3, 50.2, 33.6, 33.5, 28.4, 12.5. MS (70 eV, EI): *m*/*z* 323, 266, 210, 140, 128, 96, 84, 57.

4.2.5. 1-{[(2*R*,3a*R*,4*S*)-4-*tert*-Butoxyhexahydropyrrolo[1,2-*b*]-isoxazol-2-yl]methyl}-5-fluoropyrimidine-2,4(1*H*,3*H*)-dione 6b

The reaction of nitrone **5** (470 mg, 3.0 mmol) and allylbase **2b** (561 mg, 3.3 mmol) was carried out for 56 h thus obtaining, after purification by FCC, the pure compound **6b** (746 mg, 76%,). R_f = 0.20. White solid; mp 74–75 °C. $[\alpha]_D^{25} = -138$ (*c* 0.445, $CHCl_3$). δ_H (400 MHz, 25 °C, $CDCl_3$) 7.53 (d, 1H, =CH, *J* = 5.8 Hz), 4.36–4.28 (m, 1H, 2-H), 4.09 (dd, 1H, CH_2N_{base}, *J* = 14.1, 2.5 Hz), 3.86 (quintet, 1H, 4-H, *J* = 3.6 Hz), 3.51 (dt, 1H, 3a-H, *J* = 7.7, 3.6 Hz), 3.46 (dd, 1H, CH_2N_{base}, *J* = 14.1, 9.4 Hz), 3.37–3.22 (m, 2H, 6-H × 2), 2.31 (ddd, 1H, 3-H, *J* = 12.9, 7.2, 3.6 Hz), 2.27–2.14 (m, 2H, 3-H + 5-H), 1.67 (dquintet, 1H, 5-H, *J* = 13.1, 3.6 Hz), 1.16 (s, 9H, *tert*-Bu); δ_C (50 MHz, 25 °C, $CDCl_3$) 157.5 (d, $J_{CF}^2 = 29.4$ Hz), 149.7, 139.9 (d, $J_{CF}^1 = 233.4$ Hz), 130.6 (d, $J_{CF}^2 = 33.4$ Hz), 78.3, 74.9, 73.8, 73.1, 55.1, 50.4, 37.4, 33.7, 28.6. MS (70 eV, EI): *m*/*z* (%) 327 (23), 270 (62), 214 (20), 143 (21), 128 (79), 100 (37), 24 (40), 71 (21), 57 (100). IR ($CDCl_3$): 3383, 3176, 3076, 2977, 1702, 1663, 1471, 1377, 1365, 1318, 1243, 1187 cm^{-1}. Anal. Calcd for $C_{15}H_{22}FN_3O_4$: C, 55.04; H, 6.77; N, 12.84. Found: C, 54.73; H, 7.11; N, 12.52.

4.3. Synthesis of compounds 8–11

General procedure: A solution of the pyrrolo[1,2-*b*]isoxazolines **3a**, **3b**, **6a**, and **6b** (1 equiv) in pure trifluoroacetic acid (TFA) was kept under vigorous stirring for 3 h at room temperature. The solution was then evaporated under reduced pressure and the corresponding salt treated with a 1 M aqueous solution of NaOH (1.1 equiv). The following purification by flash chromatography (EtOAc–CH_3OH 4:1) afforded the respective homonucleoside mimetics.

4.3.1. 1-{[(2*S*,3a*S*,4*S*,5*S*)-4,5-Dihydroxyhexahydropyrrolo-[1,2-*b*]isoxazol-2-yl]methyl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione 8

Compound **3a** (320 mg, 0.809 mmol), TFA 5 mL, NaOH 1 M (0.89 mL) gave, after purification by FCC, compound **8** (148 mg, 65%). R_f = 0.23. Yellowish solid; mp 185–186 °C. $[\alpha]_D^{25} = +70$ (*c* 0.5, DMSO). δ_H (400 MHz, 25 °C, DMSO) 11.22 (br s, 1H, NH), 7.35 (q, 1H, =CH, *J* = 0.7 Hz), 5.19 (br s, 1H, OH), 5.06 (br s, 1H, OH), 4.35–4.25 (m, 1H, 2-H), 3.84 (dd, 1H, CH_2N_{base}, *J* = 14.2, 3.6 Hz), 3.76 (m, 1H, 5-H), 3.68 (t, 1H, 4-H, *J* = 4.3 Hz), 3.61 (dd, 1H, CH_2N_{base}, *J* = 14.2, 7.4 Hz), 3.33–3.27 (m, 1H, 3a-H), 3.31 (dd, 1H, 6-H, *J* = 12.0, 5.9 Hz), 2.75 (dd, 1H, 6-H, *J* = 12.0, 6.4 Hz), 2.18 (ddd, 1H, 3-H, *J* = 12.4, 6.4, 3.8 Hz), 1.96 (dt, 1H, 3-H, *J* = 12.4, 8.9 Hz), 1.72 (d, CH_3, *J* = 0.7 Hz); δ_C (50 MHz, 25 °C, DMSO) 164.6, 151.2, 142.5, 108.4, 81.8, 76.1, 74.0, 70.9, 61.5, 49.4, 37.5, 12.6. IR (KBr): 3287, 3159, 3073, 3018, 2934, 2822, 1702, 1697, 1666,

1649, 1460, 1432, 1371, 1305, 1261, 1210, 1139, 1091 cm^{-1}. Exact mass calcd for $C_{12}H_{18}N_3O_5$ [MH^+]: 284.1243. Found: 284.1241.

4.3.2. 1-{[(2*S*,3a*S*,4*S*,5*S*)-4,5-Dihydroxyhexahydropyrrolo-[1,2-*b*]isoxazol-2-yl]methyl}-5-fluoropyrimidine-2,4(1*H*,3*H*)-dione 9

Compound **3b** (165 mg, 0.413 mmol), TFA 3 mL, NaOH 1 M (0.454 mL) gave, after purification by FCC, compound **9** (82 mg, 69%). R_f = 0.24. Yellowish waxy solid. $[\alpha]_D^{25} = +42$ (*c* 0.71, DMSO). δ_H (400 MHz, 25 °C, DMSO) 7.84 (d, 1H, =CH, *J* = 6.8 Hz), 4.36–4.28 (m, 1H, 2-H), 3.84 (dd, 1H, CH_2N_{base}, *J* = 14.1, 3.8 Hz), 3.77 (dt, 1H, 5-H, *J* = 4.6, 6.1 Hz), 3.69 (t, 1H, 4-H, *J* = 4.6 Hz), 3.62 (dd, 1H, CH_2N_{base}, *J* = 14.1, 7.4 Hz), 3.34–3.28 (m, 1H, 3a-H), 3.31 (dd, 1H, 6-H, *J* = 11.8, 5.8 Hz), 2.77 (dd, 1H, 6-H, *J* = 11.8, 6.4 Hz), 2.19 (ddd, 1H, 3-H, *J* = 12.4, 6.4, 3.9 Hz), 1.98 (ddd, 1H, 3-H, *J* = 12.4, 9.1, 7.8 Hz); δ_C (50 MHz, 25 °C, DMSO) 158.5 (d, $J^2_{CF} = 24.7$ Hz), 150.6, 139.8 (d, $J^1_{CF} = 229.4$ Hz), 131.0 (d, $J^2_{CF} = 33.5$ Hz), 81.9, 76.3, 73.9, 70.9, 61.5, 49.6, 37.3. IR (KBr): 3500–3200 (br s), 2952, 2840, 1731, 1697, 1681, 1650, 1632, 1536, 1434, 1381, 1248, 1206, 1141 cm^{-1}. Exact mass calcd for $C_{11}H_{15}FN_3O_5$ [MH^+]: 288.0996. Found: 288.0991.

4.3.3. 1-{[(2*R*,3a*R*,4*S*)-4-Hydroxyhexahydropyrrolo[1,2-*b*]-isoxazol-2-yl]methyl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione 10

Compound **6a** (750 mg, 2.32 mmol), TFA (10 mL), NaOH 1 M (2.55 mL) gave, after purification by FCC, compound **10** (421 mg, 68%). R_f = 0.27. Colorless oil. $[\alpha]_D^{25} = -122$ (*c* 0.635, CH_3OH). δ_H (400 MHz, 25 °C, CD_3OD) 7.41 (q, 1H, =CH, *J* = 1.0 Hz), 4.31 (m, 1H, 2-H), 4.11 (dt, 1H, 4-H, *J* = 5.6, 3.0 Hz), 3.97 (dd, 1H, CH_2N_{base}, *J* = 14.3, 3.2 Hz), 3.62 (dd, 1H, CH_2N_{base}, *J* = 14.3, 8.1 Hz), 3.60–3.55 (m, 1H, 3a-H), 3.37–3.28 (m, 1H, 6-H), 3.22 (ddd, 1H, 6-H, *J* = 12.0, 7.2, 3.0 Hz), 2.31–2.11 (m, 3H, 3-H + 3-H + 5-H), 1.85 (br s, 3H, CH_3), 1.68 (dquintet, 1H, 5-H, *J* = 13.3, 3.0 Hz); δ_C (50 MHz, 25 °C, CD_3OD) 165.4, 151.6, 142.8, 109.1, 77.0, 75.4, 73.3, 54.8, 49.6, 36.7, 32.5, 10.9. IR (neat): 3500–3100 (br s), 3056, 2943, 2814, 1671 (br s), 1473, 1430, 1370, 1322, 1249, 1181 cm^{-1}. Exact mass calcd for $C_{12}H_{18}N_3O_4$ [MH^+]: 268.1294. Found: 268.1296.

4.3.4. 1-{[(2*R*,3a*R*,4*S*)-4-Hydroxyhexahydropyrrolo[1,2-*b*]-isoxazol-2-yl]methyl}-5-fluoropyrimidine-2,4(1*H*,3*H*)-ione 11

Compound **6b** (450 mg, 1.38 mmol), TFA (8 mL), NaOH 1 M (1.51 mL) gave, after purification by FCC, compound **11** (270 mg, 72%,). R_f = 0.21. Colorless oil. $[\alpha]_D^{25} = -93$ (*c* 1.36, CH_3OH). δ_H (400 MHz, 25 °C, CD_3OD) 7.79 (d, 1H, =CH, *J* = 6.3 Hz), 4.87 (br s, 1H, OH), 4.37–4.29 (m, 1H, 2-H), 4.12 (dt, 1H, 4-H, *J* = 5.5, 3.0 Hz), 3.97 (dd, 1H, CH_2N_{base}, *J* = 14.4, 3.2 Hz), 3.62 (dd, 1H, CH_2N_{base}, *J* = 14.4, 8.3 Hz), 3.62–3.56 (m, 1H, 3a-H), 3.35–3.28 (m, 1H, 6-H), 3.24 (ddd, 1H, 6-H, *J* = 13.1, 7.3, 3.0 Hz), 2.32–2.11 (m, 3H, 3-H + 3-H + 5-H), 1.69 (ddt, 1H, 5-H, *J* = 13.3, 6.1, 3.0 Hz); δ_C (50 MHz, 25 °C, CD_3OD) 158.0 (d, $J^2_{CF} = 30.1$ Hz), 150.0, 139.7 (d, $J^1_{CF} = 230.3$ Hz), 130.8 (d, $J^1_{CF} = 33.4$ Hz), 77.1, 75.3, 73.4, 54.9, 49.8, 36.9, 32.6. IR (neat): 3500–3100 (br s), 3075, 2952, 2824, 1692 (br s), 1436, 1379, 1246, 1205, 1136 cm^{-1}. Exact mass calcd for $C_{11}H_{15}FN_3O_4$ [MH^+]: 272.1043. Found: 272.1044.

4.4. Synthesis of compounds 12–15

General procedure: A suspension of the pyrrolo[1,2-*b*]isoxazolines **8**–**11** (1 equiv), Pd on charcoal (10%) (0.05 equiv), and TFA (1 equiv) in CH_3OH was kept under vigorous stirring at room temperature in a hydrogen atmosphere. The suspension was then filtered through Celite© and the resulting solution evaporated under reduced pressure. The following purification by flash chromatography (EtOAc–CH_3OH 4:1 with 1% of TFA) afforded the respective pyrrolidine nucleoside mimetics as TFA salts.

4.4.1. (2*S*,3*S*,4*S*)-3,4-Dihydroxy-2-[(2*S*)-2-hydroxy-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propyl]pyrrolidinium trifluoroacetate 12

A mixture of compound **8** (100 mg, 0.353 mmol), TFA (27.2 μL, 0.353 mmol), Pd on charcoal (10%) (18.8 mg, 0.018 mmol) in CH_3OH (2 mL), hydrogenated for 24 h gave, after purification by FCC, compound **12** (128 mg, 91%). R_f = 0.35. White solid; mp = 212–213 °C. $[\alpha]_D^{25} = -4$ (*c* 0.435, DMSO). δ_H (400 MHz, 25 °C, CD_3OD) 7.36 (q, 1H, =CH, *J* = 1.0 Hz), 4.18 (m, 1H, 4-H), 4.04–3.95 (m, 2H, 3-H + 2′-H), 3.88 (dd, 1H, 3′-H, *J* = 14.0, 3.6 Hz), 3.69–3.63 (m, 1H, 2-H), 3.59 (dd, 1H, 3′-H, *J* = 14.0, 8.1 Hz), 3.44 (dd, 1H, 5-H, *J* = 11.9, 3.9 Hz), 3.28 (d, 1H, 5-H, *J* = 11.9 Hz), 2.10–2.02 (m, 1H, 1′-H), 1.98–1.89 (m, 1H, 1′-H), 1.87 (d, 3H, CH_3, *J* = 1.0 Hz); δ_C (50 MHz, 25 °C, CD_3OD) 164.5, 161.3 (q, $J^2_{CF} = 33.4$ Hz), 150.9, 141.8, 116.8 (q, $J^1_{CF} = 289.3$ Hz), 108.3, 78.5, 73.8, 64.9, 62.8, 52.6, 49.9, 33.9, 10.0. IR (KBr): 3371, 3318, 3131, 3051, 2944, 2896, 2826, 1688, 1677 (br s), 1645, 1425, 1351, 1246, 1100 cm^{-1}. Exact mass calcd for $C_{12}H_{20}N_3O_5^+$ [cation]: 286.1404. Found: 286.1400.

4.4.2. (2*S*,3*S*,4*S*)-3,4-Dihydroxy-2-[(2*S*)-2-hydroxy-3-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propyl]pyrrolidinium trifluoroacetate 13

A mixture of compound **9** (107 mg, 0.374 mmol), TFA (28.8 μL, 0.374 mmol), Pd on charcoal (10%) (19.8 mg, 0.019 mmol) in CH_3OH (2 mL), hydrogenated for 4 h gave, after purification by FCC, compound **13** (98 mg, 65%). R_f = 0.33. Colorless oil. $[\alpha]_D^{25} = +4$ (*c* 0.595, CH_3OH). δ_H (400 MHz, 25 °C, CD_3OD) 7.77 (d, 1H, =CH, *J* = 6.3 Hz), 4.19 (m, 1H, 4-H), 4.06–3.98 (m, 2H, 3-H + 2′-H), 3.90 (dd, 1H, 3′-H, *J* = 13.9, 3.4 Hz), 3.67 (ddd, 1H, 2-H, *J* = 9.7, 4.4, 3.4 Hz), 3.59 (dd, 1H, 3′-H, *J* = 13.9, 8.3 Hz), 3.46 (dd, 1H, 5-H, *J* = 12.0, 4.1 Hz), 3.29 (br d, 1H, 5-H, *J* = 12.0 Hz), 2.07 (ddd, 1H, 1′-H, *J* = 14.7, 9.7, 3.4 Hz), 1.95 (ddd, 1H, 1′-H, *J* = 14.7, 8.9, 4.4 Hz); δ_C (50 MHz, 25 °C, CD_3OD) 161.9 (q, $J^2_{C-F} = 33.4$ Hz), 158.5 (d, $J^2_{C-F} = 25.2$ Hz), 150.3, 139.9 (d, $J^1_{C-F} = 232.7$ Hz), 130.9 (d, $J^2_{C-F} = 30.6$ Hz), 116.7 (q, $J^1_{C-F} = 292.4$ Hz), 79.4, 74.6, 65.7, 63.4, 53.5, 50.7, 34.7. IR (neat): 3500–3000 (br s), 2831, 1681 (br s), 1489, 1440, 1384, 1204, 1138 cm^{-1}. Exact mass calcd for $C_{11}H_{17}FN_3O_5^+$ [cation]: 290.1147. Found: 290.1152.

4.4.3. (2*R*,3*S*)-3-Hydroxy-2-[(2*R*)-2-hydroxy-3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propyl]pyrrolidinium trifluoroacetate 14

A mixture of compound **10** (59 mg, 0.22 mmol), TFA (16.9 μL, 0.22 mmol), and Pd on charcoal (10%) (11.5 mg, 0.011 mmol) in CH_3OH (1 mL) was hydrogenated for 18 h to give, after purification by FCC, compound **14** (79 mg, 94%). R_f = 0.30. Yellowish oil. $[\alpha]_D^{25} = -12$ (*c* 0.565, CH_3OH). δ_H (400 MHz, 25 °C, CD_3OD) 7.37 (q, 1H, =CH, *J* = 1.0 Hz), 4.23 (dt, 1H, 3-H, *J* = 5.4, 4.4 Hz), 4.08–4.00 (m, 1H, 2′-H), 3.88 (dd, 1H, 3′-H, *J* = 14.0, 3.6 Hz), 3.65–3.57 (m, 2H, 2-H), 3.62 (dd, 1H, 3′-H, *J* = 14.0, 8.1 Hz), 3.47–3.33 (m, 2-H, 5-H × 2), 2.27–2.17 (m, 1H, 4-H), 2.02–1.92 (m, 1H, 4-H), 1.86 (d, 3H, CH_3, *J* = 1.0 Hz), 1.82 (t, 2H, 1′-H × 2, *J* = 6.3 Hz); δ_C (50 MHz, 25 °C, CD_3OD) 164.5, 161.5 (q, $J^2_{CF} = 33.1$ Hz), 150.9, 141.7, 117.0 (q, $J^1_{CF} = 289.6$ Hz), 108.4, 72.8, 65.0, 62.3, 52.3, 41.9, 32.3, 30.3, 9.9. IR (neat): 3500–3100 (br s), 3050, 2952, 2849, 1681 (br s), 1471, 1430, 1356, 1247, 1225, 1081 cm^{-1}. Exact mass calcd for $C_{12}H_{20}N_3O_4^+$ [cation]: 270.1448. Found: 270.1450.

4.4.4. (2*R*,3*S*)-3-Hydroxy-2-[(2*R*)-2-hydroxy-3-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)propyl]pyrrolidinium trifluoroacetate 15

A mixture of compound **11** (81 mg, 0.30 mmol), TFA (23 μL, 0.30 mmol), Pd on charcoal (10%) (15.7 mg, 0.015 mmol) in CH_3OH (1.5 mL), hydrogenated for 2 h gave, after purification by FCC, compound **15** (89 mg, 77%,). R_f = 0.28. Pale yellow oil. $[\alpha]_D^{25} = +11$ (*c* 2.595, CH_3OH). δ_H (400 MHz, 25 °C, CD_3OD) 7.77 (d, 1H, =CH,

J = 6.2 Hz), 4.22 (dt, 1H, 3-H, J = 5.1, 4.7 Hz), 4.10–4.01 (m, 1H, 2′-H), 3.89 (dd, 1H, 3′-H, J = 14.0, 3.2 Hz), 3.66–3.57 (m, 2H, 3′-H + 2-H), 3.45–3.34 (m, 2H, 5-H), 2.28–2.18 (m, 1H, 4-H), 2.01–1.91 (m, 1H, 4-H), 1.88–1.81 (m, 1H, 1′-H); δ_C (50 MHz, 25 °C, CD_3OD) 162.2 (q, J^2_{CF} =33.8 Hz), 158.5 (d, J^2_{CF} = 26.1 Hz), 150.4, 140.0 (d, J^2_{CF} = 232.3 Hz), 130.9 (d, J^2_{CF} = 31.4 Hz), 117.1 (q, J^1_{CF} = 287.3 Hz), 73.7, 65.7, 63.0, 53.4, 42.8, 33.2, 31.2. IR (neat): 3500–3100 (br s), 3077, 2965, 2828, 1681 (br s), 1439, 1383, 1248, 1204, 1138 cm^{-1}. Exact mass calcd for $C_{11}H_{17}FN_3O_4^+$ [cation]: 274.1198. Found: 274.1200.

Acknowledgements

Authors thank MIUR (Ministry of University and Research—Rome) for financial support (PRIN: 2005032057). V.M. acknowledges the receipt of a Research fellowship (“Assegno premiante”) from the University of Florence—Dept of Organic Chemistry. Mrs. Brunella Innocenti and Mr. Maurizio Passaponti are acknowledged for technical support.

References

1. Merino, P. *Curr. Med. Chem. Anti-Infective Agents* **2002**, *1*, 389–411.
2. Chiacchio, A.; Genovese, F.; Librandolannazzo, D.; Iannazzo, V.; Merino, P.; Rescifina, A.; Romeo, R.; Procopio, A.; Romeo, G. *Tetrahedron* **2004**, *60*, 441–448.
3. Chiacchio, U.; Saita, M. G.; Crispino, L.; Gumina, G.; Mangiafico, S.; Pistarà, V.; Romeo, G.; Piperno, A.; De Clercq, E. *Tetrahedron* **2006**, *62*, 1171–1181.
4. (a) Chiacchio, U.; Corsaro, A.; Iannazzo, D.; Piperno, A.; Pistarà, V.; Rescifina, A.; Romeo, R.; Velveri, V.; Mastino, A.; Romeo, G. *J. Med. Chem.* **2003**, *46*, 3696–3702; (b) Chiacchio, U.; Balestrieri, E.; Macchi, B.; Iannazzo, D.; Piperno, A.; Rescifina, A.; Romeo, R.; Saglimbeni, M.; Sciortino, M. T.; Valveri, V.; Mastino, A.; Romeo, G. *J. Med. Chem.* **2005**, *48*, 1389–1394.
5. For the cycloaddition of pyrroline-N-oxide to vinyl nucleobases see: Procopio, A.; Alcaro, S.; De Nino, A.; Maiuolo, L.; Ortuso, F.; Sindona, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 545–550.
6. (a) Goti, A.; Cicchi, S.; Cordero, F. M.; Fedi, V.; Brandi, A. *Molecules* **1999**, *4*, 1–12; (b) Cordero, F. M.; Gensini, M.; Goti, A.; Brandi, A. *Org. Lett.* **2000**, *2*, 2475–2477; (c) Goti, A.; Cicchi, S.; Cacciarini, M.; Cardona, F.; Fedi, V.; Brandi, A. *Eur. J. Org. Chem.* **2000**, *12*, 3633–3645; (d) Cordero, F. M.; Pisaneschi, F.; Gensini, M.; Goti, A.; Brandi, A. *Eur. J. Org. Chem.* **2002**, *12*, 1941–1951.
7. (a) Richichi, B.; Cicchi, S.; Chiacchio, U.; Romeo, G.; Brandi, A. *Tetrahedron* **2003**, *59*, 5231–5240; (b) Richichi, B.; Cicchi, S.; Chiacchio, U.; Romeo, G.; Brandi, A. *Tetrahedron Lett.* **2002**, *43*, 4013–4015.
8. Adams, D. R.; Boyd, A. S. F.; Ferguson, R.; Grierson, D. S.; Monneret, C. *Nucleosides Nucleotides* **1998**, *17*, 1053–1075.
9. (a) Cicchi, S.; Goti, A.; Brandi, A. *J. Org. Chem.* **1995**, *60*, 4743–4748; (b) Cicchi, S.; Höld, I.; Brandi, A. *J. Org. Chem.* **1993**, *58*, 5274–5275.
10. De Clercq, E. *J. Med. Chem.* **2005**, *48*, 1–17.
11. Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250–6284 and references cited therein.
12. (a) McCaig, A. E.; Meldrum, K. P.; Wightman, R. H. *Tetrahedron* **1998**, *54*, 9429–9446; (b) Cicchi, S.; Crea, S.; Goti, A.; Brandi, A. *Tetrahedron: Asymmetry* **1997**, *8*, 293–301; (c) Brandi, A.; Cicchi, S.; Goti, A.; Koprowski, M.; Pietrusiewicz, K. M. *J. Org. Chem.* **1994**, *59*, 1315–1318.
13. The solubility tests carried out revealed that this salt is partially soluble in ethyl acetate and considerably in methanol and in the mixtures of these two solvents that were employed for the FCC. Unfortunately, the use of other eluents for the purification step appeared unfeasible, due to the polarity of compounds **8**–**11**.
14. In the case of compound **8**, for example, an almost equimolecular mixture with sodium trifluoroacetate is easily soluble in chloroform. On the contrary, in the absence of this salt, dimethylsulfoxide is the only solvent possible, with **8** being almost completely insoluble in other common solvents as chloroform, ethyl acetate, and methanol.
15. Both weakly and strongly basic resins were employed with some success, but with a considerable loss of the products.
16. ^{1}H NMR of the crude products showed the partial reduction of the double bond in both cases. To minimize this collateral process it is necessary to monitor by TLC the disappearance of the starting material.