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FULL PAPER

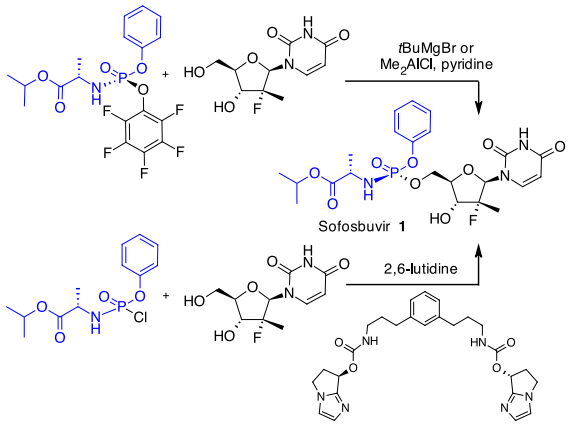
Stereoselective synthesis of Sofosbuvir through nucleoside phosphorylation controlled by kinetic resolution

Elena Cini,[a] Giuseppe Barreca, [b] Luca Carcone, [b] Fabrizio Manetti, [a] Marcello Rasparini,[b]‡ and Maurizio Taddei*[a]

Abstract: The preparation of Sofosbuvir, the potent key component of recent Hepatitis C (HCV) infection therapies, is reported here. The process is based on the dynamic kinetic resolution of the stereochemically unstable isopropyl-2-((chloro(phenoxy)phosphoryl)-amino)propanoate (**8**). The high stereoselection obtained is due to the correct choice of the protective group at 3'-OH. While ester and carbonate-based protections gave an inferior stereoselection, benzyl protection allowed phosphorylation to occur in 92:8 ratio in favour of the product with the right configuration at the P-stereogenic centre. Starting from the γ-lactone of 2-deoxy-2-fluoro-2-methyl-pentonic acid, the synthesis was accomplished in 8 steps in 40 % overall yield using commercially available reagents and without any enzymatic or chemical resolution technique.

Introduction

Sofosbuvir is an antiviral agent approved for treatment of Hepatitis C Virus (HCV) infections.[1] The remarkable activity of this molecule is related to the presence of a phosphoramide moiety in position 5' of the nucleoside. This pro-drug approach, called ProTide, allows: i) a substantial increase of the cell permeability of the molecule ii) the generation of the nucleotide monophosphate in cell after a simple metabolic transformation with subsequent increase of in vivo phosphorylation rate.[2] A comprehensive SAR study revealed that an enantiomerically pure *L* amino acid linked to the phosphorous is required for the first step of the ProTide metabolism, generating a cyclic intermediate through substitution of the aryloxy moiety bonded to the phosphorous. Final ring opening and hydrolysis of the phoshoramide produces the monophosphate that is rapidly transformed into the active triphosphate metabolite. This architecture requires a chiral phosphorous and the configuration may influence activity, metabolic transformation and even toxicity.[3] Thus, an efficient synthesis of ProTide modified nucleosides (as Sofosbuvir) must include the stereoselective formation of a P-stereogenic center. Amongst different strategies employed for ProTide syntheses, the nucleophilic substitution on a phosphorochloridate with the nucleoside nucleophile is the most common preparative method. Unfortunately, this approach gives a mixture of diasteroisomers that must be separated by chromatography or other methods, with loss of valuable material.[4] A potential solution to this approach is the transformation of the phosphorochloridate into the corresponding pentafluorophenol derivative that is sterochemically stable at the P-center.[5] After fractional crystallization, the correct isomer is subject to nucleophile substitution that occurs with almost complete inversion of configuration giving the required stereochemistry at P. In the case of Sofosbuvir (or analogue nucleotides) with a free 3'OH in the final structure, a selective reaction at the 5'OH is also required. This problem was originally approached with the use of protective groups at 3' and recently with the use of a Lewis acid that selectively activate the 5'OH for reaction with the isopropyl ((S)-(perfluorophenoxy)(phenoxy)-phosphoryl)-L-alaninate (Scheme 1).[6] Finally, a group of Merck scientists published recently the use of a chiral catalyst for the stereoselective assemble of ProTide nucleosides starting from the stereochemically unstable isopropyl-2-((chloro(phenoxy)-phosphoryl)amino)propanoate (Scheme 1). Using the unprotected nucleoside, a tailor made enantiomerically pure catalyst is required to reach a high level of regio- and stereoselectivity.[7]



Scheme 1. Most recent synthetic approaches to Sofosbuvir (ref 5-7). In blue the ProTide appendage.

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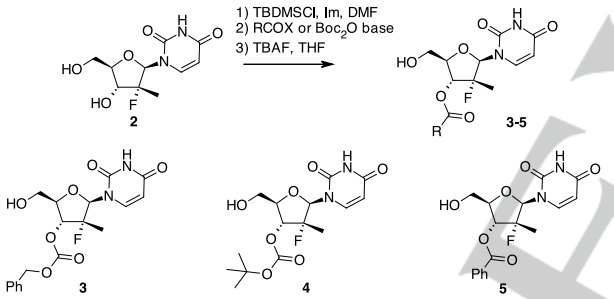
Following our interest in the synthesis of relevant active pharmaceutical ingredients (APIs),[8,9] engaged in developing a convenient preparation of Sofosbuvir, we decided to investigate the possibility to carry out a dynamic resolution of the racemic phosphochloridate using the enantiomerically pure nucleoside itself as the selector.

The working hypothesis was to study the influence of the size of the protective group at 3'OH and the nature of the base employed to activate the OH on phosphorylation stereoselectivity. Some examples of phosphorylation of protected nucleosides have been previously described,[10–12] although with low selectivity. However, a systematic study on the influence of the protection at the 3' position has been never reported and also the influence of the base employed on the stereoselectivity has been not deeply investigated.

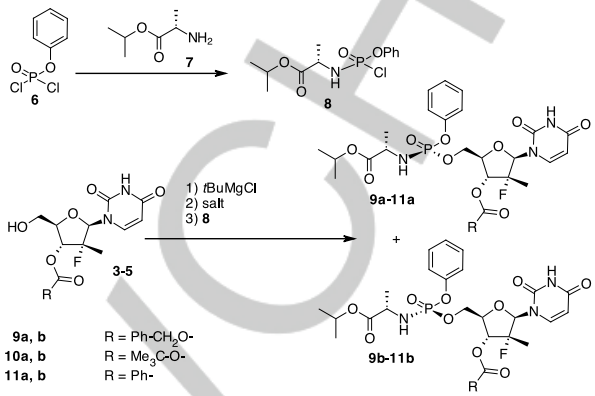
Results and Discussion

Thus, nucleosides **3-5** were prepared starting from nucleoside **2** through the classic transient protection at 5' with TBDMS, further acylation of the 3' OH and final desilylation with TBAF (Scheme 2).[11]



Scheme 2. Protection at 3'OH of nucleoside **2**. For **3**, X = N-hydroxysuccinimide, for **5**, X = Cl.

As deprotection of compound **9** (Scheme 3) was anticipated to be the easiest to perform, the optimization of reaction conditions started on nucleoside **3** (Scheme 3). Phosphorylation of **3** was attempted with chloro(phenoxy)phosphoryl)amino)propanoate **8** prepared *in situ* from phenyl dichlorophosphate **6** and the *iso*-propyl ester of *L*-alanine (Scheme 3). After activation of the 5'-OH of **3** with *t*-BuMgCl, different salts were added in the reaction mixture in order to study the influence of the cation (and probably the resulting aggregation) on the stereoselectivity. As reported in Table 1, the presence of different salts did not have a dramatic influence on the stereoselectivity of the reaction. Increase of the cation size, anion size or the presence of ammonium salt in solution did not improve the selectivity that resembled that of the reaction without additives. The use of LiCl (added) or the use of the complex *i*PrMgCl LiCl, the so-called Turbo-Grignard,[13] gave best results in terms of selectivity and conversion (entries 2 and 10 in Table 1).



Scheme 3. Phosphorylation of 3'-protected nucleosides **3-5**.

Table 1. Influence of the presence of salts in phosphorylation of **3**

Entry	Salt[a]	Conversion (%)[b]	dr **(9a**:**9b)**[c]
1	-	94	65:35
2	LiCl	85	73:27
3	NaCl	67	65:35
4	KCl	96	64:26
5	CsF	46	65:35
6	LiF	86	66:34
7	CsCl	70	67:33
8	KBr	96	65:35
9	KI	44	63:37
10	LiCl (complex)	90	83:17
11	$ZnCl_2$	41	62:38

a] Reaction conditions: [b] Determined respect to unreacted nucleoside by HPLC analysis. [c] Determined by HPLC using isopropyl ((S)-bis-(phenoxy)phosphoryl)-L-alaninate as internal standard.

The more hindered Boc derivative **4** gave comparable results in terms of conversion and selectivity (Figure 1). The benzoyl derivative **5** gave better conversion again with the preformed LiCl complex with the Grignard, but always an almost equimolar amount of the two diasteroisomers (Figure 1). Although not completely satisfactory, the Cbz protection at 3' and the nucleoside activation at 5' with the Turbo-Grignard were encouraging enough to further investigate other benzyl protections.[9] Surprisingly, the 3'O-benzyl derivative (**15** in Scheme 5) has been never reported before, we prepared it, following a synthetic approach comparable to that applied for compounds **3-5**

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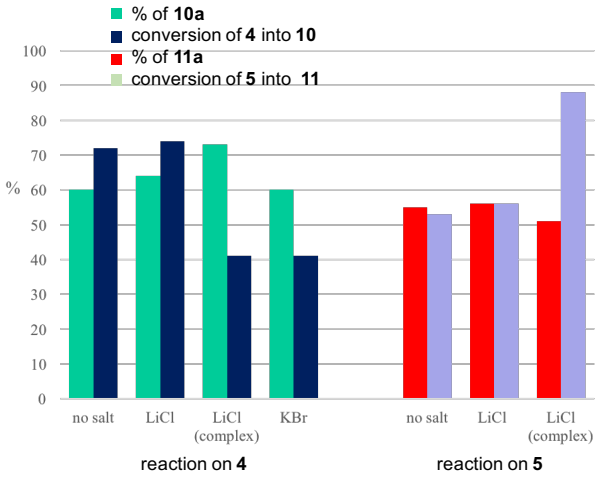
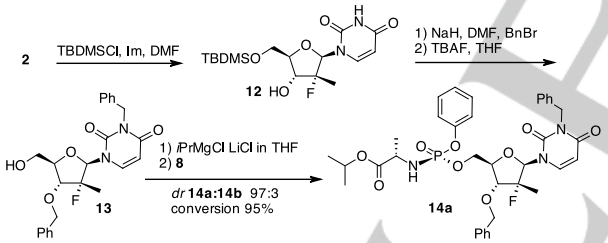


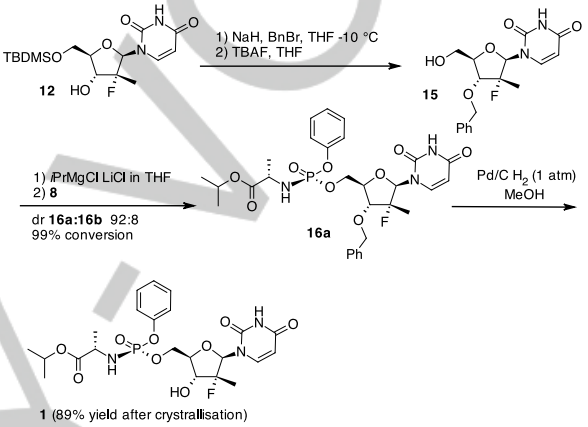
Figure 1. Influence of the presence of salts in phosphorylation of **4** and **5**.

Unfortunately, after silylation of the 5'OH, NaH in DMF was employed in order to activate the 3'OH and exclusively the dibenzyl derivative **13** was obtained even after increasing the ratio between **12** and NaH /benzyl bromide (Scheme 4). However, when compound **13** was submitted to our best reaction conditions, the phosphorylated compound **14** was obtained with high conversion and a very high dr (97:3 in favor of the correct diastereoisomer **14a**). [9]



Scheme 4. Preparation and phosphorylation of the dibenzyl derivative **13**

Unfortunately, deprotection of **14a** to Sofosbuvir **1** was troublesome and conversion to pure **1** was possible only after a multistep purification from several by-products.[9]
Thus, we tried to find a method for the selective protection of the OH in 3' without affecting the uracil NH. We came across a paper that describes the selective 3'-O- or 3-N-protection of 5'-O-TBDMS-thymidines using aprotic polar solvents with low or high dielectric constant, respectively.[14] The reaction was described under ultrasound or microwave activation, but we were pleased to discover that reacting **12** with 3 eq of NaH in THF and 1 eq of benzyl bromide at -10°C for 12 h, exclusively the monobenzylated compound **15** was obtained in 63% overall yield. The correct position of the benzyl was independently established by X-ray analysis of the product (SI), confirming the decisive solvent effect in nucleoside alkylation.
Compound **15** was then submitted to reaction with **8** after activation with *i*PrMgCl LiCl complex and compound **16** was obtained with very high conversion (99%) and a still excellent dr (92:8). This product was readily deprotected with H_2 over Pd/C in MeOH to give product **1** in almost quantitative yield (Scheme 5). The product obtained in 89 % yield after a single crystallization, showed the same spectroscopic and physical characteristics of the original Sofosbuvir.



Scheme 5. Transformation of 3'-benzylderivative **15** into Sofosbuvir.

Although very efficient, this procedure might be considered less economical in terms of atoms employed and number of steps with respect to the catalytic procedures that, starting from **2**, do not use protection at 3'OH.[6,7,15] However, all the published syntheses of compound **2**,[15,16] described starting from commercially available lactone **17** (Scheme 6) requires the use of protections. A first full protection of the two hydroxyl groups with benzoyl chloride is required (**18**) before performing the introduction of the nucleobase as 4-benzoylcytosine (Scheme 6).[12] Thus, after reduction of the carbonyl of **18** and further acetylationof the OH of **19**, the nucleobase **20** is silylated to product **21** prior the reaction, to give fully protected compound **22**. The protective groups of **22** are removed with ammonia in MeOH and finally the 4-benzoylcytosine is transformed into uracil with hot acetic acid[16] to give nucleoside **2** (Scheme 6, reactions inside the red frame). Compound **2** may then be transformed in a single step using selective phosphorylation at 5'OH cited in Scheme 1 to give Sofosbuvir in a 7 steps procedure from **17** .[5–7] Consequently, as protection at the OH is required for nucleobase insertion, we decided to introduce also our different protective groups at 3' and 5' OHs on lactone **18**, obtaining compound **23** in 67% yield (Scheme 6). Reduction of the lactone followed by acetylation and cytosine introduction gave acceptable yields (71% overall) of product **25**. The acid environment required for transformation of the benzoyl-cytosine into uracil allowed the contemporary

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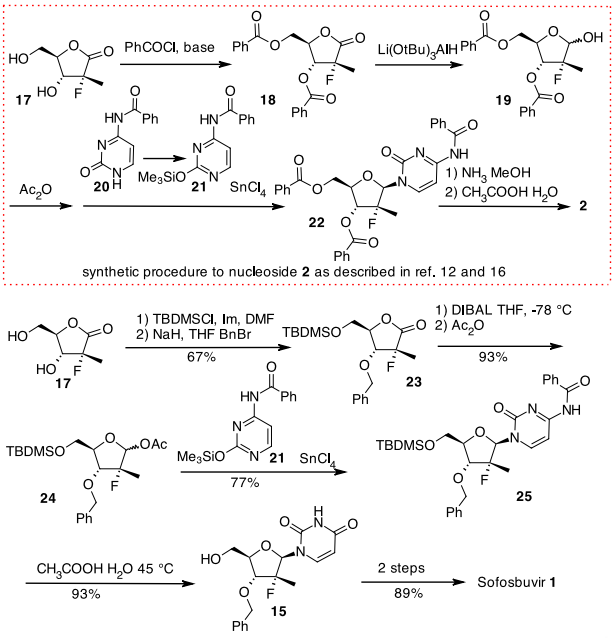
removal of the TBDMS group at 5' position, giving compound **15** in good yields.



Scheme 6. Comparison of our Sofosbuvir synthesis with the previously described one (in the red dotted line frame) starting from the common intermediate lactone **17**.

Finally, the transformation into Sofosbuvir, as described in Scheme 6, required additional two reactions. Overall, our approach, based on the dynamic resolution of the easily accessible chloride **8**, employs commercially available (low cost) reagents and provides Sofosbuvir in 40% isolated yield starting from available lactone **17**. The process is realized in 8 steps, only one step more (the debenzylation at 3')[17] if compared with the previous synthesis based on the selective phosphorylation at 5' with a tailor made chiral catalyst.[7]

In order to rationalize the variation in stereoselectivity observed changing the protection at 3'OH, phosphorylation of **15** was monitored by HPLC analysis in different conditions. The selectivity did not change during reaction time, with a value of 95:5 at 5% conversion. The amount of **8** employed had no influence on selectivity; however, in the presence of less than 1 eq of **8**, no trace of **16ab** was detected in the reaction medium. A computational protocol consisting in B3LYP/6-31G** calculations using THF and PBF as the solvent and solvation model, respectively, in combination with a systematic pseudo Monte Carlo conformational routine[7] was attempted, but the locations of Mg and Li ions made results difficult to rationalize. We observed that the presence of a carbonyl in position 3' of the nucleoside stabilizes an intramolecular complex with Mg, while, with a benzyl ether, this arrangement is not possible. Probably, with benzyl ether **15** an intermolecular chelated transition state with **8** might be formed, favoring the formation of the $P_{(R)}$ configuration on the final phosphorylated product.

Conclusions

Phosphoramidate nucleotides are the key constituents of the ProTide approach to prodrugs successfully employed in therapies against viral pathologies as HCV infections. While previous procedures are based on standard racemate resolution, use of chiral auxiliaries or complex catalysts specifically designed for the process, we have developed a stereoselective method for the introduction of the P-stereogenic center via kinetic dynamic resolution of the stereochemically labile chloro(phenoxy)-phosphoryl)amino)propanoate,[18] using the nucleoside protected at 3' with a benzyl group as chiral selector. Only this expedient is required to obtain high stereoselectivity and all our synthesis proceeds with commercially available reagents. Extension of this process to other therapeutically relevant ProTide nucleotides are currently under investigation.

Experimental Section

Benzyl ((*2R,3R,4R,5R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-yl) carbonate (3). General procedure. To a solution of (2'*R*)-2'-deoxy-2'-fluoro-2'-methyluridine **2** (1.87 g,7.2 mmol) in anhydrous pyridine (15 mL) at 0 °C under N_2, imidazole (0.62 g, 9,2 mmol) and *tert*-butyldimethylsilyl chloride (1.20 g, 7.85 mmol) were added. The reaction mixture was stirred at r.t. for 12 h and then quenched with methanol (5 mL). After 1 h of additional stirring at r.t. the solvent was removed under vacuum. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc 1:4 v/v) to give **12** (2.46 g) in 93% yield. This product was dissolved in dry CH_2Cl_2 (50 mL) and *N*-(benzyloxycarbonyloxy)succinimide (1.97 g, 7.92 mmol) was added under N_2. The reaction mixture was cooled to 0 °C and DMAP (176 mg, 1.44 mmol.) followed by Et_3N (1.42 mL, 10.8 mmol,) was added. The reaction mixture was slowly warmed to room temperature and stirred for 16 h. A 1 M HCl solution was added (20 mL), the phases separated, the aqueous phase extracted with CH_2Cl_2 and the collected organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc 8:2) to give the O-Cbz protected product (1.11 g, 75% yield) as a colourless oil. This compound was solubilized in THF (22 mL), treated with TBAF (2.07 g, 3.51 mmol,) and stirred for 3 h at room temperature. The solvent was evaporated and the product purified by column chromatography (EtOAc) to give **3** (1.45 g, 68%) as a white solid (m.p. 153-154 °C) with the same spectroscopic data described in the literature.[11] Anal. calcd for $C_{18}H_{19}FN_2O_7$: C 54.82, F 4.82, H 4.86, N 7.10, O 28.40; found: C 54.79, H 4.83, N 7.07.

***tert*-Butyl ((*2R,3R,4R,5R*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-yl) carbonate (4):** Compound **4** was prepared following the general procedure above using in Boc_2O in place of CbzOSu; 43% overall yield as a white solid m.p. 159-161 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.95 (s, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 6.15 (d, *J* = 18.2 Hz, 1H), 5.73 (d, *J* = 8.1 Hz, 1H), 4.94 (dd, *J* = 22.3, 9.3 Hz, 1H), 4.16 (d, *J* = 9.2 Hz, 1H), 4.05 (dd, *J* = 12.7, 3.8 Hz, 1H), 3.75 (d, *J* = 12.7 Hz, 1H), 3.33 (s, 1H), 1.56 – 1.23 (m, 12H) . ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.66, 153.11, 150.57, 140.00, 102.94, 99.80, 89.31, 84.39, 79.80, 73.18 (d, *J* = 15.9 Hz), 59.40, 27.56 (3C), 16.98 (d, *J*

= 25.0 Hz). Anal. calcd for $C_{15}H_{21}FN_2O_7$: C 50.00, F 5.27, H 5.87, N 7.77, O 31.08; found: C 49.05, H 5.85, N 7.74

(2*R*,3*R*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-2-(hydroxymethyl)-4-methyltetrahydrofuran-3-yl benzoate (5): Compound **5** was prepared following the general procedure above using benzoyl chloride in place of CbzOSu: 47% overall yield as a white solid m.p. 248-250 °C. ^{1}H NMR (400 MHz, DMSO) δ = 11.51 (s, 1H), 8.00 (m, 3H), 7.68 (t, *J* = 7.4 Hz, 1H), 7.54 (m, 2H), 6.10 (d, *J* = 19.1 Hz, 1H), 5.72 (d, *J* = 8.1 Hz, 1H), 5.55 – 5.18 (m, 2H), 4.28 (d, *J* = 8.9 Hz, 1H), 3.94 – 3.52 (m, 2H), 1.33 (d, *J* = 23.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 164.92, 162.90, 150.58, 139.86, 134.01, 129.58 (2C), 128.92 (2C), 128.56, 102.49, 100.30 (d, *J* = 183 Hz), 88.97, 79.94, 71.74 (d, *J* = 13 Hz), 58.67, 17.39 (d, *J* = 24 Hz). Anal. calcd for $C_{17}H_{17}FN_2O_6$: C 56.04, F 5.21, H 4.70, N 7.69, O 26.35; found: C 56.00, H 4.67, N 7.66,

3-Benzyl-1-((2*R*,3*R*,4*R*,5*R*)-4-(benzyloxy)-3-fluoro-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (13): A solution of **12** (500 mg, 1.34 mmol prepared as described in the above general procedure) in dry DMF (3 mL) was cooled to 0 °C . NaH (110 mg of 60% dispersion in mineral oil, 2.7 mmol) was slowly added having care to maintain the temperature below 5 °C. After 10 min of stirring at 5°C, benzyl bromide (0.320 mL, 2.7 mmol) was added dropwise. When the addition was complete, the temperature was raised to 25 °C and the mixture was kept under stirring until the conversion was complete, monitoring by TLC with petroleum ether /EtOAc 7:3 (about 15 h). The reaction mixture, cooled to 0 °C, was diluted with diethyl ether. Phases were separated, and the aqueous phase was extracted with diethyl ether; the organic phases were combined and dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash column chromatography (petroleum ether/ EtOAc 7:3) to give O,N-di-Bn protected product (638 mg, 86% yield) as a colourless oil that was solubilized in THF (15 mL) and treated with TBAF (543 mg, 1.72 mmol,) for 3 h at room temperature. The solvent was evaporated, and the product purified by column chromatography (EtOAc) to give **13** as a white solid (320 mg, 62%). m.p. 97-99 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 6.6 Hz, 1H), 7.49 – 7.15 (m, 10H), 6.05 (d, J = 17.0 Hz, 1H), 5.75 (d, J = 8.1 Hz, 1H), 5.14-5.02 (AB system, 2H), 4.77-4.65 (AB system, 2H), 4.18 – 3.87 (m, 3H), 3.71 (dd, J = 12.2, 4.2 Hz, 1H), 1.94 (s, 1H), 1.26 (d, J = 22.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.87, 150.60, 137.58, 136.82, 136.05, 128.42 (2C), 128.21 (2C), 128.08, 128.00 (2C), 127.91 (2C), 127.27, 101.95, 99.6 (d, J = 182.9 Hz), 90.52 (d, J = 28.0 Hz), 80.13, 76.59 (d, J = 16.3 Hz), 73.67, 59.42, 43.79, 17.00 (d, J = 25.5 Hz). HRMS (ESI): calcd. for $C_{24}H_{25}FN_2NaO_5$ $[M+Na]^+$ 463,1645; found 463,1649.

1-((2*R*,3*R*,4*R*,5*R*)-4-(Benzyloxy)-3-fluoro-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (15). A solution of **12** (1.85 g, 4.95 mmol prepared as described in the above general procedure) in dry THF (20 mL) was cooled to -10 °C. NaH (593 mg of the 60% dispersion in mineral oil 14.8 mmol) was added over 10 min in portions of approximately 50 mg each. After stirring the mixture at -10 °C for 30 min, benzyl bromide (0.588 mL, 4.95 mmol) was added dropwise. When the addition was complete, the temperature was raised to r.t. and kept under stirring for 16 hours. The reaction mixture, cooled to 0 °C, was diluted with diethyl ether (40 mL) and water (20 mL) was slowly added. The phases were separated, the aqueous phase extracted with Et_2O and the collected organic phases were dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc 8:2) to give O-Bn protected product (1.33 g, 2.87 mmol, 58% yield) as a colourless oil that was solubilized in THF (15 mL) and treated with TBAF (1.36 g, 4.30 mmol, 1.5 equiv) for 3 h at room temperature. The solvent was evaporated and the product purified by column chromatography (EtOAc) to give **15** (634 mg, 63%) as a white solid m.p. 180-182 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.45 (s, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.49 – 7.13 (m, 5H), 5.97 (d, *J* = 19.1 Hz, 1H), 5.66 (d, *J* = 8.0 Hz, 1H), 5.38 (s, 1H), 4.69 (s, 2H), 4.06-3.84 (m, 3H), 3.67 (d, *J* = 12.5 Hz, 1H), 1.28 (d, *J* = 22.5 Hz, 3H) . ^{13}C NMR (100 MHz, DMSO) δ 162.88, 150.62, 139.77, 138.00, 128.33 (2C), 127.87 (3C), 102.16, 100.47 (d, *J* = 181.3 Hz), 88.48 (d, *J* = 39 Hz), 80.92, 77.78 (d, *J* = 16.3 Hz), 73.14, 58.83, 17.23 (d, *J* = 17.23 Hz). Anal. calcd for $C_{17}H_{19}FN_2O_5$: C 58.28, F 5.42, H 5.47, N 8.00, O 22.83; found: C 58.23, H 5.45, N 7.97.

(2*S*)-Isopropyl 2-(((((2*R,3R,4R,5R*)-3-(benzyloxy)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (16). To a flask provided with a mechanical stirrer, reflux condenser, thermometer and under N_2, (*L*)-alanine (5.0 g, 56.1 mmol) was mixed with a solution of hydrogen chloride in *i*-propanol (1 1 .1 % w/w, 73.8 g, 224.5 mmol). The reaction mixture was heated to boiling (80-85 °C) for 4 hours. Once the conversion was complete, (monitoring in TLC by eluting with 7:3 ethanol-water and developing with ninhydrin) the solution was concentrated to residue in vacuum to give (*L*)-alanine isopropyl ester hydrochloride as a dense oil (9.4 g quantitative yield). To a flask provided with mechanical stirrer, reflux condenser, thermometer and under N_2, (L)-alanine isopropyl ester hydrochloride (9.4 g, 56.1 mmol) was mixed with methyl *t*-butyl ether (80 mL) and the resulting mixture was stirred at room temperature until a homogeneous suspension was obtained. The mixture was then cooled to -55 °C and Et_3N (13.0 g, 128.5 mmol) was added, looking to maintain the system at a temperature below -50 °C. At the end of the addition, the mixture was maintained at -55 °C and a solution of phenyl dichlorophosphate (10.8 g, 51.2 mmol) in methyl *t*-butyl ether (9.5 mL) was added, keeping the temperature below -50 °C. The reaction mass was stirred at -55 °C until the conversion was complete (about 2 hours). The reaction mixture was heated to -20 °C and filtered under N_2 atmosphere. The filtrate was stored at -20 °C under N_2 supposing a quantitative formation of chloride **8**. Inside a flask equipped with mechanical stirrer, reflux condenser, thermometer and under N_2, compound **15** (8.9 g, 25.4 mmol) was dissolved in dry THF (140 mL). This solution was cooled to -25 °C and *i*-propylmagnesium chloride lithium chloride complex 1:1 (1.3 M in THF, 39 mL, 50.7 mmol), was slowly added making sure that the temperature did not exceed -20 °C. When the addition was complete, the reaction mixture was stirred at -20 °C for 2 h and then a solution of the previously prepared phosphorylchloride **8** was slowly added over about 2 h, keeping the temperature below -20 °C. Once the conversion was complete (approximately 2 h), the reaction mixture was poured in a mixture of isopropyl acetate (170 mL), water (90 mL) and acetic acid (4 mL) cooled to 0 °C. The mixture was slowly heated to room temperature (20-25 °C), and the phases were separated. The organic phase was washed with water and concentrated to residue under vacuum, removing the residual solvents by co-evaporation with methanol, having care to maintain the temperature of the hating bath below 40 °C. As amorphous residue was obtained (15.7 gr, 99% conversion) that was analysed by HPLC; column: Symmetry® C18 (4.6 mm x 25 mm, 5 μm); eluent: H_2O (+0.1% H_3PO_4)/CH_3CN 75:25; flow: 1 mL/min; detector: UV lamp, λ 254 nm; conversion: 98%, r.d.: 93:7. The sample was crystallized from isopropyl acetate/methanol to give the analytical sample. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.59 – 6.99 (m, 11H), 6.09 (d, *J* = 18.8 Hz, 1H), 5.63 (d, *J* = 8.1 Hz, 1H), 4.94 (dt, *J* = 12.6, 6.3 Hz, 2H), 4.82 – 4.40 (m, 3H), 4.36 – 3.69 (m, 4H), 1.52–0.92 (m, 12H). HRMS (ESI) calcd. for $C_{29}H_{35}FN_3O_9PNa$ 642.1993 $[M+Na]^+$; found 642.1990.

Sofosbuvir (1). Product **16** (16.9 g, 25.4 mmol) was dissolved in methanol (200 mL) and treated with H_2 (1 atm) at r.t. using Pd/C as a catalyst (10% w/w, 50% wet, 2.7 g, 1.3 mmol). After 12 h of stirring, the conversion was complete, and the mixture was filtered on a Celite pad to remove the Pd and the filtered cake washed with methanol. The solution was concentrated to residue under vacuum and residual methanol was removed by repeated co-evaporation with dichloromethane. 14.1 g of residue are obtained (quantitative conversion). After crystallisation from

dichloromethane /acetonitrile, compound **1** (12.5 gr) was obtained in 89% yield. HPLC analysis of the residue (carried out by means of a Symmetry® C18 column (4.6 mm x 25 mm, 5 μιη) as eluent H_2O (+0.1 % H_3PO_3)/CH_3CN 45:55 at 1 mL/min and UV 254 nm detector) shows a quantitative conversion and a diastereomeric purity higher than 99%. m.p. 97-99 °C. ^{1}H NMR (400 MHz, DMSO) δ 11.47 (s, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.36-7-32 (m, 2H), 7.28 – 7.09 (m, 2H), 6.22 – 5.92 (m, 2H), 5.81 (d, *J* = 4.5 Hz, 1H), 5.51 (d, *J* = 7.9 Hz, 1H), 5.04 – 4.61 (m, 1H), 4.51 – 4.25 (m, 1H), 4.22-4-20 (m, 1H), 3.97-3.75 (m, 3H), 1.25 – 1.11 (m, 12H). ^{13}C NMR (100 MHz, DMSO) δ 172.64, 162.76, 150.77, 150.47, 139.59, 129.68 (2C), 124.61, 120.11(2C), 102.29, 100.32 (d, *J* = 179.9 Hz), 88.42, 79.51, 71.57 (d, *J* = 16.8 Hz), 68.02, 64.77, 49.83, 21.42 (2C), 19.81 (d, *J* = 6.3 Hz), 16.56 (d, *J* = 25.1 Hz). ^{31}P NMR (162 MHz, DMSO-d6) δ 4.82. Anal. calcd for calcd. for $C_{22}H_{29}FN_3NaO_9P$: C 49.91, F 3.59, H 5.52, N 7.94, O 27.20, P 5.85; found C 49.96, H 5.50, N 7.97.

(3*R*,4*R*,5*R*)-4-(Benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-fluoro-3-methyldihydrofuran-2(3H)-one (23): To a solution of **17** (900 mg, 5.49 mmol) in dry CH_2Cl_2 (10 mL) was added dry pyridine (1.02 mL, 12.6 mmol) under N_2 and the reaction mixture was cooled to 0 °C. Imidazole (403 mg, 6.31 mmol) and *tert*-butyldimethylsilyl chloride (910 mg, 6.04 mmol) dissolved into dry CH_2Cl_2 (3 mL) were added. The reaction mixture was slowly warmed to r.t. and stirred for 16 h then a 1 M HCl solution was added (10 mL), the phases separated, the aqueous phase extracted with CH_2Cl_2 and the collected organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc 7:3) to give the 3' silylated product (1.34 g, 4.82 mmol, 88% yield) as a white solid. This product was solubilized in THF (40 mL), cooled to –10 °C and NaH (578 mg of 60% dispersion in mineral oil, 14.46 mmol) was added in portions over 10 min and the mixture was stirred at -10 °C for 30 min, then benzyl bromide (573 μL, 4.82 mmol,) was added dropwise. When the addition was complete, the mixture was slowly warmed to r.t. and kept under stirring for 16 h. To the reaction mixture, cooled to 0 °C, diethyl ether and water were added, the phases separated, the aqueous phase extracted with diethyl ether, the collected organic phases dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc 9:1) to give **23** (1.35 g, 76% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.29 (m, 5H), 4.77 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 11.7 Hz, 1H), 4.42 (dt, *J* = 6.5, 2.3 Hz, 1H), 4.05 (dd, *J* = 8.0, 4.5 Hz, 1H), 3.95 (dd, *J* = 12.2, 2.4 Hz, 1H), 3.73 (dd, *J* = 12.2, 2.4 Hz, 1H), 1.54 (d, *J* = 23.2 Hz, 3H), 0.85 (s, 9H), 0.05 (d, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.91 (d, *J* = 22.1 Hz), 136.93, 128.65 (2C), 128.45, 128.19 (2C), 91.56 (d, *J* = 184.6 Hz), 81.71, 76.85 (d, *J* = 17.1 Hz), 73.74, 60.48, 25.79 (3C), 19.44 (d, *J* = 24.9 Hz), 18.28, -5.40, -5.52. HRMS (ESI): calcd. for $C_{19}H_{29}FNaO_4Si$ $[M + Na]^+$ 391,1717; found 391,1711.

***(3R,4R,5R)*-4-(Benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-3-fluoro-3-methyltetrahydrofuran-2-yl acetate (24).** Protected lactone **23** (220 mg, 0.60 mmol) was dissolved in dry THF (4 mL) and the solution was cooled to -78 °C under a nitrogen atmosphere. DIBAL 1.0 M in THF (1.20 mL, 1.20 mmol, 2 equiv) dropwise. After 3 h, the reaction was complete at TLC analysis. Water (300 μL was added), temperature was raised to room temperature and kept under stirring for 30 min. Dry Na_2SO_4 (1 g) was added and after 30 min of stirring at r.t., the mixture was filtered on Celite, the filtrate was evaporated to give the crude lactol that was solubilized in CH_2Cl_2 and treated with DMAP (7 mg, 0.06 mmol,) and acetic anhydride (357 μL, 3.78 mmol) and the reaction was stirred at room temperature for 16 h. The reaction was concentrated, and the residue was purified by flash column chromatography (petroleum ether/EtOAc 8:2) to give the acetate **24** as colorless oil (230 mg, 93%) 4:1 mixture of anomers as determined by NMR in $CDCl_3$. Data for the major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.13 (m, 5H), 6.01 (d, J = 11.2 Hz, 1H), 4.70 (m, 2H), 4.14 (dd, J = 7.7, 3.4 Hz, 1H), 3.99 (dd, J = 24.1, 8.1 Hz, 1H), 3.89 – 3.65 (m, 2H), 2.03 (s, 3H), 1.37 (d, J = 22.0 Hz, 3H), 0.90 (s, 9H), 0.06, (s, 3H), 0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.11, 137.72, 128.49 (2C), 128.09, 128.03 (2C), 99.75 (d, J = 179.3 Hz), 98.51 (d, J = 36.3 Hz), 79.16 (d, J = 15.6 Hz), 73.78, 62.33, 25.91 (3C), 21.11, 18.38, 16.83 (d, J = 24.6 Hz), -5.33, -5.45. HRMS (ESI): calcd. for $C_{21}H_{33}FNaO_5Si$ [M+Na]+ 435,1979; found 435,1984.

***N*-(1-((3*R*,4*R*,5*R*)-4-(Benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)-methyl)-3-fluoro-3-methyltetrahydrofuran-2-yl)-2-oxo-1,2-dihydro-pyrimidin-4-yl)benzamide (25) and conversion to nucleoside 15:** The silylated cytosine base was prepared by refluxing a suspension of N-benzoylcytosine (125 mg, 0.585 mmol) and ammonium sulfate (4 mg, 0.029 mmol, 0.05 equiv.) in hexamethyldisilazane (1.3 mL) for 2 h and concentrating the resulting solution by vacuum distillation, followed by drying the residue under vacuum (0.2 mmHg) for 2 h at r.t. The oily residue was dissolved in chlorobenzene (2 mL). To this solution was added the acetate **24** (160 mg, 0.39 mmol) and neat tin tetrachloride (199 μL, 1.72 mmol). After stirring under a nitrogen atmosphere for 2 h at r.t., the reaction was heated to 70 °C for 19 h. The reaction mixture was cooled to 0 °C and solid sodium bicarbonate (0.94 g) and EtOAc (50 mL) were added. To the stirred solution was slowly added water (20 mL) (caution: vigorous evolution of carbon dioxide). The mixture was vigorously stirred for 30 min at r.t., the suspension was filtered and the collected solid was washed with EtOAc (12 mL). The solution was concentrated, and the residue was purified by flash column chromatography (petroleum ether/EtOAc 6:4) to give **25** as colourless oil (169 mg, 0.26 mmol, 77%). Data for the major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 7.2 Hz, 1H), 7.92 (d, *J* = 6.7 Hz, 2H), 7.61 – 6.99 (m, 10H), 6.36 (d, *J* = 17.6 Hz, 1H), 4.75 – 4.57 (m, 2H), 4.20 (dd, *J* = 18.2, 10.6 Hz, 2H), 3.88-3-99 (m, 2H), 1.34 (d, J = 20.0 Hz, 3H), 0.98 (s, 9H), 0.16 (s, 6H). The so obtained compound **25** was suspended in 80% aqueous AcOH (5 mL) and heated at reflux for 12 h. The clear solution was concentrated, and the residue was purified by flash column chromatography (petroleum ether/ethyl acetate 6:4) to give the nucleoside **15** (confirmed by H^{1}NMR and HPLC) as a white solid (57 mg, 93%).

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Keywords: antiviral agents • kinetic resolution • nucleotides • API synthesis • protecting groups

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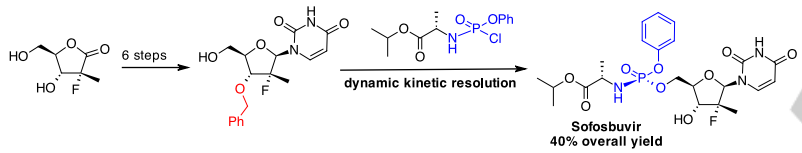

A new method for the introduction of the P-stereogenic center via dynamic kinetic resolution of the stereochemically labile phosphorylchloride, employs the nucleoside protected at 3' with a benzyl group as the chiral selector. Only this expedient is required to obtain high stereoselectivity in an 8-step synthesis of the anti-HCV drug Sofosbuvir.

***API synthesis**

*Elena Cini, Giuseppe Barreca, Luca Carcone, Fabrizio Manetti, Marcello Rasparini, and Maurizio Taddei**

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Stereoselective synthesis of Sofosbuvir through nucleoside phosphorylation controlled by kinetic resolution