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Synthesis of N-1 and ribose modified inosine analogues on solid support

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Abstract—Herein, we report the synthesis and the use of new *N*-1-dinitrophenyl-inosine based solid supports, in which the C-2 of the purine base is strongly activated toward the attack of N-nucleophiles. The synthesized supports, binding the nucleoside by a 5′-*O*-monomethoxytrityl function, have been used to accomplish the synthesis of a small library of N-1 alkylated inosine and AICAR derivatives. In addition, cleavage of the 2′-3′ ribose bond of N-1 alkylated inosine derivatives anchored to the supports allowed to prepare a new set of N-1 alkylated-2′,3′-secoinosine derivatives in high yields.

Solid phase reactions have been extensively applied to a variety of organic reactions, furnishing an easy and rapid procedure for modifying functional groups and/or to conjugate specific molecules to target products. Solid phase reactions facilitate the synthesis of large libraries of compounds endowed with a series of molecular diversity motifs around a single core scaffold. Screening libraries of synthetic compounds for biological activity is currently the most widely used approach toward drug discovery. Nucleosides are biomolecules possessing a pivotal role in the metabolism. In fact, they are involved, as tri-phosphate derivatives, in the nucleic acid replications and in a very wide number of interactions with enzymes, structural proteins and other biological targets of therapeutic importance. In this light, it is not surprising that the most important antiviral drugs[1] currently used in therapy, and other active compounds exhibiting antineoplastic,[2] antibiotic, and antifungal properties,[3] have been developed starting from this class of molecules. Recently, the interest toward large nucleoside libraries has emerged as an important synthetic goal to allow a wide range of biological screenings. A variety of solid phase combinatorial strategies have been reported for the preparation of nucleoside and small oligonucleotide analogues libraries. In these approaches, both the chemical stability and the position of the linkage with the polymeric support play an important role in the selection of the chemical treatments allowing the nucleobase or sugar–phosphate modifications. For example, a solid support binding uridine or thymidine by the acid labile 5′-*O*-trityl function has been used to prepare libraries of N-4-alkylated cytidine derivatives.[4] Another recently reported nucleoside-bearing support has exploited the alkaline labile 5′-*O*-succinyl linkage to bind the 6-chloro-2-nitro-inosine to synthesize a set of N-6- or N-2-alkylated adenosine derivatives.[5] Also the 2′,3′-acetal linkage has been used to bind nucleosides to a solid matrix which has been employed to introduce chemical diversities both on the base (C-6 of purine and C-4 of pyrimidine) and on the 5′-ribose position.[6] In another approach, recently proposed by some of us, a purine or pyrimidine nucleoside was anchored to the solid support by the N-3 or N-1 base position, respectively, through a *N*-alkyl-β-thioether function[7,8] which resulted stable to both the acidic and basic conditions. The above supports have been used to synthesize 2′-,3′-ribose modified nucleoside or nucleotide analogues. Furthermore, controlled pore glass (CPG) supports, loading

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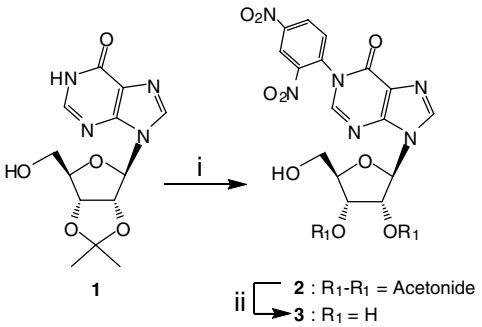


Scheme 1. Reagents and conditions: (i) DNCB (2.2 equiv), K_2CO_3 (2.0 equiv) 2 h, 80 °C; (ii) $HCOOH/H_2O$ (6:4, v/v), 4 h, rt.

5′-DMT-nucleosides by the classical 3′-succinyl linkage[9–11] or by the 3′-acyloxyaryl phosphate linker,[12] have been successful exploited to prepare very large nucleic-acid-bases (NAB™) libraries of 5′-phosphoramidate nucleoside derivatives and nucleic acid fragments to be tested in their antiviral activity.

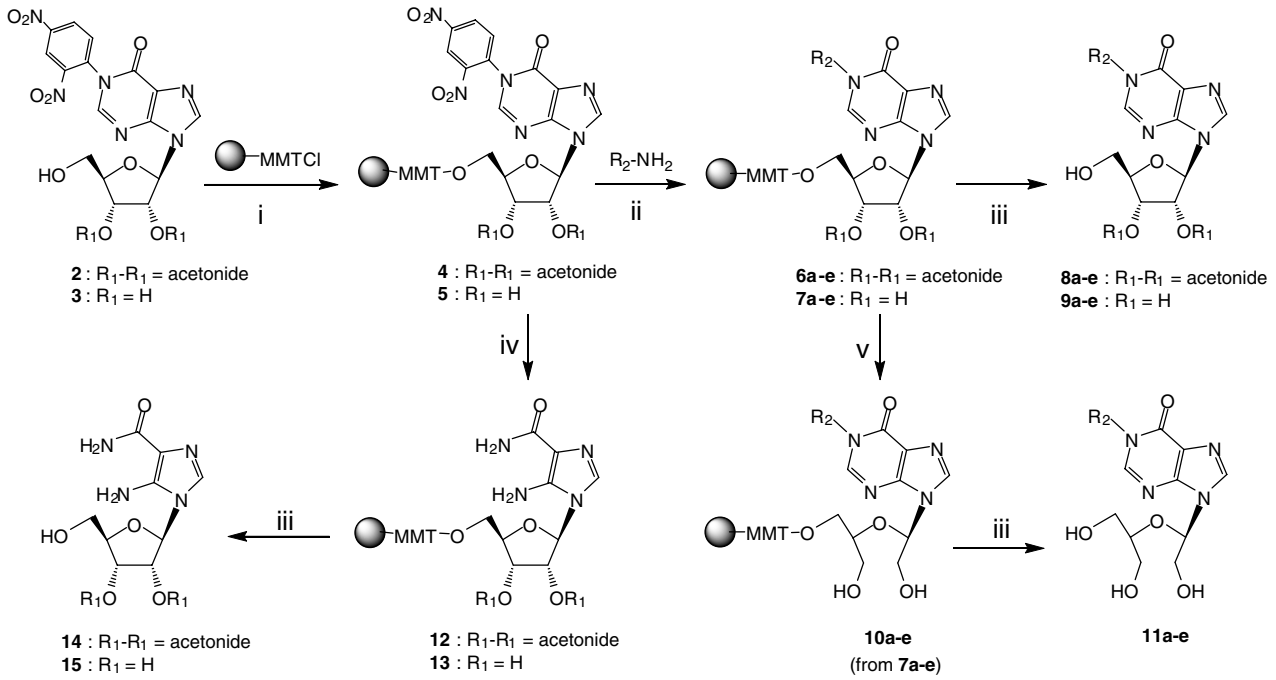
In an effort to enlarge the nucleoside chemical reactivity on the solid phase and consequently the number of accessible structurally diverse analogues, we report here the synthesis and exploitation of the new nucleoside functionalized supports **4** and **5** which bind the *N*-1-dinitrophenyl-inosine derivatives **2** or **3** through the 5′-*O*-trityl function. These supports have been employed in the synthesis of the N-1 substituted inosine **8a**–**e**, **9a**–**e**, the related 2′,3′-seconucleoside derivatives **11a**–**e** and the 5-aminoimidazole-4-carboxamide riboside (AICAR) derivatives **14** and **15**. The here proposed solid phase strategy is based on our previous studies on the C-2 reactivity of the *N*-1-dinitrophenyl-2′-deoxyinosine toward N-nucleophiles[13,14] that allowed to obtain N-1 substituted inosine and AICAR derivatives. In particular, according to the reported reaction mechanism, when a strong electron-withdrawing group (such as the 2,4-dinitrophenyl, nitro,[15] or arylsulfonyl[16] group) is attached to the N-1 atom of the hypoxanthine ring, the C-2 carbon becomes electrophilic enough to react with amino nucleophiles (R_2–NH_2). This leads to the opening of the six membered ring through the cleavage of the (N-1)–(C-2) bond. The successive fast ring re-closure, favoured by the loss of the 2,4-dinitroaniline as the leaving group, furnished the N-1 alkyl inosine derivatives. It is to be noted that as a consequence of the purine rearrangement, the endocyclic N-1 atom is substituted by the nitrogen atom of the nucleophilic reactant. This purine reactivity has also been used by others to introduce modified purine bases into oligonucleotides.[17] Thus, to obtain a small library of N-1 alkyl inosine derivatives, we bound the 1-(2,4-dinitrophenyl)-2′,3′-*O*-isopropylideneinosine **2**, or the corresponding unprotected inosine derivative **3** (Scheme 1 and 2), to the commercially available polystyrenemonomethoxytrityl chloride (MMTCl) resin by 5′-*O*-trityl ether linkage. Inosine derivative **2** was synthesized by reaction of the commercially available 2′,3′-*O*-isopropylidene inosine **1** with 2,4-dinitrochlorobenzene (DNCB) essentially as previously described.[18] The 2′-3′ deprotected inosine derivative **3**[†] was obtained treating **2** with aqueous formic acid (90% yield). The reaction of the MMTCl polystyrene resin (1.3 mequiv/g) with **2** or **3** in anhydrous pyridine at room temperature and in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) afforded support **4** or **5**, respectively, in almost quantitative yield. The structure and the loading of supports **4** and **5** were confirmed by NMR analysis and quantitative UV experiments, on the released inosine derivatives **2** and **3** obtained by treating the support with 2% TFA in DCM (8 min, rt). Supports **4** and **5** were then reacted with several N-nucleophiles (R_2–NH_2, Table 1, entries a–e) to give supports **6a**–**e** and **7a**–**e**, respectively. In a typical reaction, 100 mg (0.13 mmol) of support **4** (or **5**), swollen in DMF, was left in contact with the R_2–NH_2 nucleophile (5.0 mmol) in 1.5 mL of DMF under shaking (8 h, at 50 °C). After washings with DMF and MeOH, the support was dried under reduced pressure and the reaction yield was evaluated detaching the nucleoside material from a weighted amount of resin. The reaction of **4** or **5** with ethylenediamine (Table 1, entry f) furnished, as expected,[14] supports **12** and **13** bearing the 2′-3′-isopropylidene-AICAR and the AICAR, respectively, in almost quantitative yields. The structure and the loading of supports **6**, **7**, **12**, and **13** were ascertained by analyzing with HPLC,[‡] ^{1}H NMR (Table 1) and MS[§] the corresponding detached N-1 alkyl inosine derivatives **8a**–**e**, **9a**–**e** as well as AICAR **14** and its derivative **15**. The purity and the yield of the above detached nucleosides, confirmed by HPLC analysis and quantitative UV experiments, resulted to be always over 90–95%, (Table 1). Starting from 20 mg of solid support (**4** or **5**), and considering an average molecular weight of 300 g/mol, 6–7 mg of each nucleoside derivative could be obtained in 90–98% purity, thanks to the almost quantitative reaction yields.

The second goal of this work was to combine the set of the N-1 alkylated inosine derivatives (from supports **7**) with a ribose modification. As an example we have examined the well known 2′,3′-oxidative cleavage of the ribose moiety generally carried out by reaction with metaperiodate followed by reduction of the di-aldehyde derivative which leads to 2′,3′-seconucleosides.[19] In a typical reaction, supports **7a**–**e** (100 mg, 0.13 mmol) were left in contact with a solution of $NaIO_4$ (1.3 mmol) in DMF/H_2O (1.5 mL, 1:1, v/v) and shaken for 12 h at 60 °C. The resulting support, after washings with DMF and EtOH, was treated with $NaBH_4$ (2.6 mmol) in

[†] Product **3**, as a 1:1 mixture of atropisomers at N-1 phenyl bond; ^{1}H NMR (400 MHz, CD_3OD): δ 9.05 (s, 1H, H-3 DNP); 8.77 (dd, 1H, H-5 DNP); 8.49, 8.49, 8.48, 8.40 (s's, H-2 and H-8); 8.02 (dd, 1H, H-6 DNP); 6.10 and 6.12 (d's, 0.5 H each, H-1′); 4.69 and 4.64 (dd's, 0.5 H each, H-2′); 4.35 (m, 1H, H-3′); 4.15 (m, 1H, H-4′); 3.88 and 3.77 (m, 1H each, H-5′).

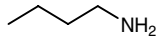
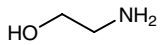
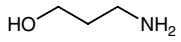
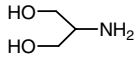
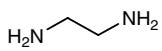
[‡] HPLC analyses: RP18 analytic column eluted with a linear gradient of CH_3CN in 0.1 M TEAB (pH 7.0, from 0% to 60% in 60 min, flow 1.0 mL/min).

[§] ESI MS data *m*/*z* (calcd): **9a** 325.2 ($M+H^+$) (324.1); **9b** 313.2 ($M+H^+$) (312.1); **9c** 327.1 ($M+H^+$) (326.1); **9d** 355.1 ($M+H^+$) (354.1); **9e** 343.2 ($M+H^+$) (342.1); **11a** 349.1 ($M+Na^+$) (326.2); **11b** 337.2 ($M+Na^+$) (314.1); **11c** 351.2 ($M+Na^+$) (328.1); **11d** 379.2 ($M+Na^+$) 356.2; **11e** 367.2 ($M+Na^+$) 344.1; **15** 259.0 ($M+H^+$) (258.1).



Scheme 2. Reagents and conditions: (i) compound **2** or **3** (1.5 equiv) in pyridine (1.5 mL/250 mg of resin), DMAP (0.2 equiv), 24 h, rt; (ii) R_2–NH_2 (38.0 equiv) in DMF, 8 h, 50 °C; (iii) TFA 2% solution in DCM; (iv) EDA/DMF (1:1, w/w) 8 h, 50 °C; (v) $NaIO_4$ (10 equiv) in DMF/H_2O (1:1, v/v), 12 h, 60 °C; resin washings and treatment with $NaBH_4$ (20 equiv) in EtOH, 2 h, rt.

Table 1. Reactions of the supports **4** and **5**; products **8**, **9**, **11**, **14**, and **15**

Entry	R_2–NH_2	**8**, **9**, **14**, **15** Yield[a] (%)	**9a–e** and **15** ^{1}H NMR[b]		**11a–e**	^{1}H NMR[b]	
			H-2; H-8; H-1′	R_2 Moiety	Yield[c] (%)	H-2; H-8; H-1′ (*seco*)	R_2 Moiety
a		**8** (98) **9** (98)	8.41; 8.36; 6.02	4.12; 1.76; 1.40; 0.98	**11a** (85)	8.30; 8.26; 6.04	4.00; 1.75; 1.38; 0.96
b		**8** (96) **9** (95)	8.42; 8.26; 6.01	4.19; 3.82	**11b** (85)	8.28; 8.24; 6.04	4.20; 3.83
c		**8** (98) **9** (96)	8.41; 8.32; 6.02	4.20; 3.60; 1.98	**11c** (84)	8.31; 8.26; 6.03	4.21; 3.60; 1.98
d		**8** (98) **9** (98)	8.40; 8.35; 6.02	4.12; 3.56; 1.81; 1.59; 1.42	**11d** (82)	8.33; 8.27; 6.03	4.11; 3.55; 1.79; 1.58; 1.43
e		**8** (92) **9** (90)	8.38; 8.32; 6.00	3.98 ($2CH_2OH$); 3.92 (CH)	**11e** (75)	8.29; 8.04; 6.05	4.02 ($2CH_2OH$) 3.95 (CH)
f		**1** (98) **1** (98)	8.04; 5.66		NT	NT	

[a] Starting from resin **4** or **5** respectively; the yield is almost coincident with the purity degree of the product.
[b] 400 MHz, (CD_3OD) significative protons at ppm.
[c] Starting from resin **5**; the yield (before HPLC purification) is almost coincident with the purity degree of the product.

1.5 mL of EtOH and shaken for 2.0 h at rt. After washings, the obtained supports **10a–e** were dried under reduced pressure and analyzed by detaching the nucleoside material by TFA treatment. HPLC analyses indicated that the 2′,3′-secoinosine derivatives **11a–e** were obtained in 75–85% yield and with almost the same purity degree. The sole side-products, obtained in 15–25% yields, were the corresponding uncleaved nucleoside. All the structures were confirmed by ^{1}H NMR (Table 1) and MS analyses.[§] When the above oxidative cleavage was performed in EtOH/H_2O (various solvent ratios and temperatures were used), the corresponding seco-nucleosides were obtained in lower yields, most probably due to the negligible swelling of the polystyrene matrix in these solvent mixtures.

In conclusion, we have reported the synthesis of new *N*-1-dinitrophenyl-inosine based solid supports (**4** and **5**) in

which the nucleosides are anchored to a MMT–polystyrene resin by the 5′ position. Supports **4** and **5** were converted into the N-1 alkylated inosine supports (**6** and **7**) and AICAR derivatives supports (**12** and **13**) in very high yields, by reacting at C-2 position of the purine base with R–NH_2 nucleophiles. Detachment of the nucleosidic material from the above supports furnished, in high yields and purities, small libraries of N-1 alkylated inosines (**8a–e**, **9a–e**) and AICAR derivatives (**14** and **15**) (90–98%) possessing the ribose moiety either protected or unprotected at the 2′-3′-hydroxyl functions. In a further solid phase reaction, we have combined the set of the N-1 alkylated inosine of supports **7a–e** with the cleavage of the 2′,3′ ribose bond. These reactions furnished the new group of solid supports **10a–e** bearing the corresponding acyclo-nucleosides. Supports **10a–e** released, under acidic conditions, the N-1 alkylated-2′,3′-secoinosine derivatives **11a–e** in good yields and purity (75–85%). In our opinion, supports **6**, **7**, **10**, **12**, and **13** bearing nucleoside derivatives with high purity degree can be fruitfully utilized in a combinatorial manner to obtain a number of further derivatizations/conjugations both on the 1-(ω-hydroxyalkyl) function and/or on the ribose or secoribose moieties. Further studies are currently in progress in this direction to obtain new large libraries of nucleoside analogues that will be screened against a wide range of biological assays.

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