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A Versatile Route to C-6 Arylmethyl-Functionalized *S*-DABO and Related Analogues

Marco Radi,[†] Lorenzo Contemori,[†] Daniele Castagnolo,[†] Raffaella Spinosa,[†] José A. Esté,[‡] Silvio Massa,[†] and Maurizio Botta*,[†]

Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via Alcide de Gasperi 2, I-53100, Siena, Italy, and Retrovirology Laboratory irsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autonoma de Barcelona, E-08916 Badalona, Spain

botta@unisi.it

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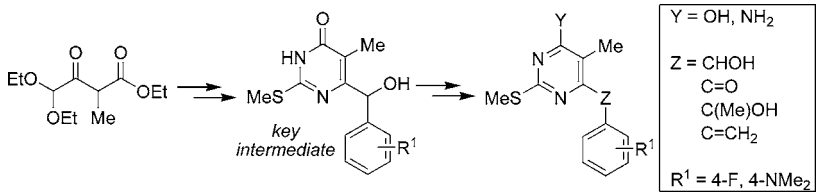
ABSTRACT



Since their discovery in 1992, 3,4-dihydro-2-alkoxy-6-benzyl-4-oxypyrimidines (DABOs) have been subjected to many structural modifications in order to obtain better non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of AIDS. Herein, we report a straightforward and versatile route for the synthesis of novel C-6 arylmethyl-functionalized *S*-DABO, a poorly explored class of derivatives. Finally, biological evaluation of the synthesized derivatives led to the identification of a promising anti-HIV-1 lead compound.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent essential components in first-line anti-HIV-1 therapy due to their good tolerability and lack of association with lipodystrophy generally associated with the administration of protease inhibitors.[1] NNRTIs include more than 30 structurally different classes of molecules, such as nevirapine, TIBO, HEPT, TNK-561, ITU, DATA, and DAPY.[2] These compounds bind to a specific allosteric site of HIV-1 RT near the polymerase site and interfere with reverse transcription by altering either the conformation or mobility of RT, thereby leading, with only one exception,[3] to a noncompetitive inhibition of the enzyme. Among the NNRTIs reported to date, DABO analogues (Figure 1) have been the object of great interest since their discovery in 1992[4] and have led to the identification of highly potent compounds against both HIV-1 RT wild type (wt) and drug-resistant mutants.[5] In the

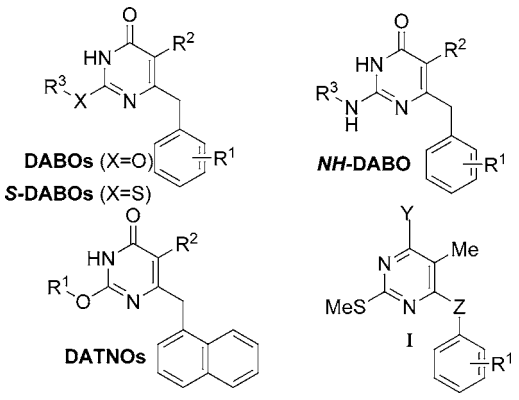


Figure 1. Common DABO analogues and target compound **I**.

[†] Università degli Studi di Siena.
[‡] Universitat Autonoma de Barcelona.

(1) Barbaro, G. Barbini, G. *Chemotherapy* **2006**, *52*, 161−165.
(2) (a) Boone, L. R. *Curr. Opin. Invest. Drugs* **2006**, *7*, 128−135. (b) Barbaro, G.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. *Curr. Pharm. Des.* **2005**, *11*, 1805−1843.
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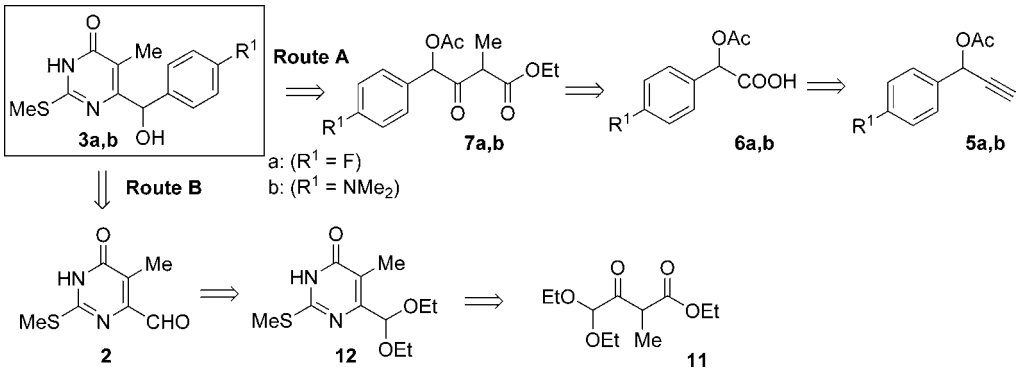
Scheme 1. Retrosynthetic Approach for the Key Intermediates **3a**,**b**

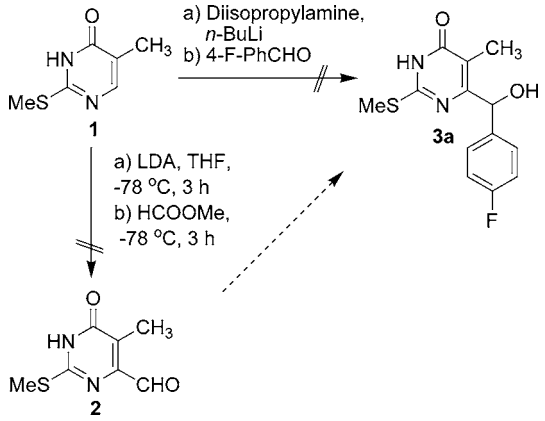


search for agents able to target the NNRTIs drug-resistant mutants, many different modifications have been performed on the pyrimidinone scaffold of the DABO-family during the past 25 years: (i) introduction of different chains at position C-2; (ii) substitution of the hydrogen in C-5 with bulkier groups; (iii) introduction of different substituents on the phenyl ring at position C-6; and (iv) substitution of the phenyl ring in C-6 with different aromatic or heteroaromatic moieties.[6] However, few modifications of the arylmethyl carbon at the C-6 position have been reported so far, and it has been recently shown by Ji et al.[7] that this kind of functionalization led to potent anti-HIV-1 DABOs, although only biological data for the HIV-1 (wt) infected MT-4 cells were disclosed.[8]

Herein, we report a straightforward and versatile approach for the synthesis of C-6 arylmethyl-functionalized *S*-DABO analogues (general structure **I**, Figure 1) accessible by consecutive functionalization of the C-6 hydroxy group in the key intermediates **3a**,**b** (Scheme 1). The identification of a lead compound belonging to a new family of *S*-DABO cytosine analogues is also disussed.

In our original idea, the key intermediate **3a** could be obtained by two alternative pathways, namely the direct lithiation in C-6 and reaction with the appropiate aldheyde[9] or passing through the C-6-formyl intermediate **2** (Scheme 2).[10] However these approaches were unsuccessful even starting from the corresponding N^3-benzyl- and *O*-benzyl-protected derivatives of compound **1**. Two different synthetic routes were then planned for the synthesis of the key intermediates **3a**,**b** (Scheme 1): according to route A, **3a**,**b** could be obtained after cyclization of *S*-methylisothiourea (SMT) with the β-ketoesters **7a**,**b** and final *O*-deacetylation. The intermediates **7a**,**b** could be achieved after condensation of potassium ethyl 2-methyl malonate with **6a**,**b**, which could in turn be obtained via oxidation of **5a**,**b**. According to route B, the key intermediates **3a**,**b** could be obtained via Grignard reaction on the aldehyde **2**, which could in turn be obtained after deprotection of the acetal **12** resulting from the cyclization of SMT with the β-ketoester **11**.

Scheme 2



Following the approach described in route A, 4-fluorobenzaldehyde **4a** was converted in compound **5a** via reaction with ethynylmagnesium bromide and subsequent protection of the α-hydroxy group as acetyl derivative (Scheme 3). Compound **5a** was then oxidized to the corresponding carboxylic acid **6a**, which was activated as imidazolide and then reacted with potassium ethyl 2-methylmalonate to give the β-ketoester **7a**. Unfortunately, the subsequent condensation of **7a** with SMT did not afford the expected pyrimidinone **8a**, while the lactone **9a** was obtained as the only product. This synthetic pathway could represent, however, an alternative approach for the synthesis of 3,5-disubstituted

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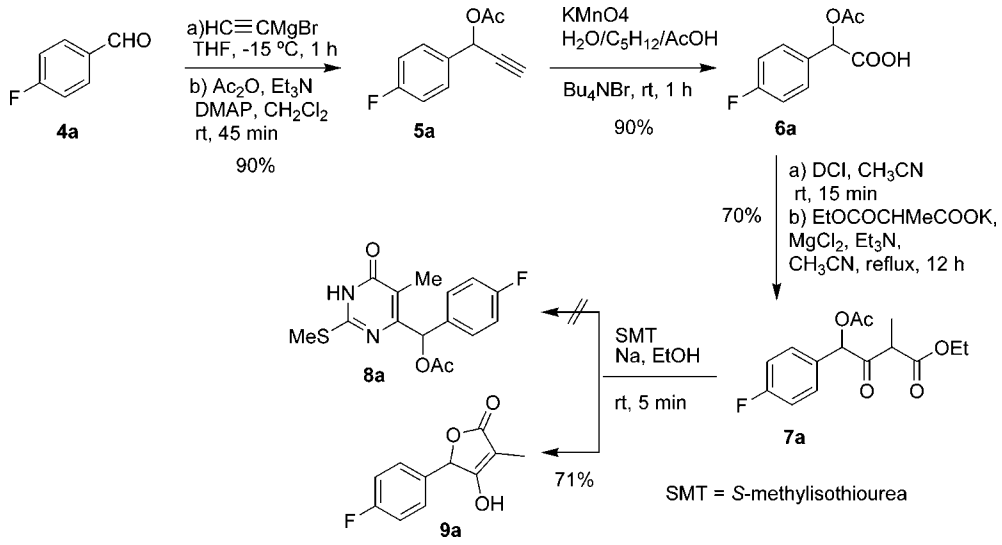
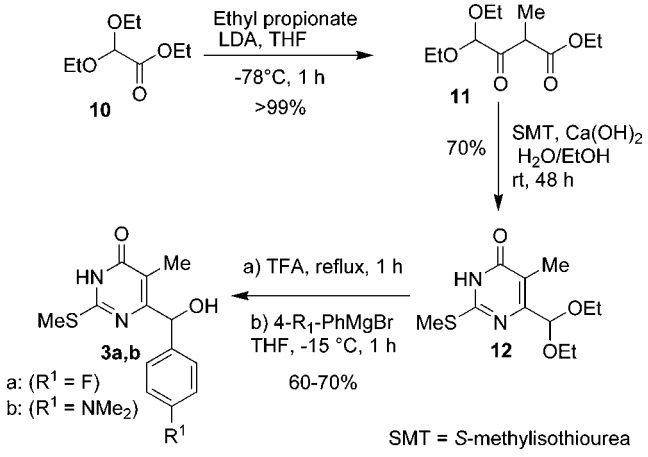
Scheme 3



tetronic acid derivatives.[11] Different protecting groups were then used, in place of the acetyl, to mask the hydroxy moiety in order to overcome the intramolecular cyclization of compound **7a**, but the desired pyrimidinone **8a** was never obtained. Following route B (Scheme 4), mixed Claisen

Scheme 4



condensation between the ethyl diethoxyacetate **10** (bearing a masked formyl group) and ethyl propionate gave quantitatively the β-ketoester **11** which was then submitted to cyclization reaction with SMT to give the pyrimidinone **12** in 70% yield. Deprotection of **12** in refluxing trifluoroacetic acid gave quantitatively the C-6 formyl derivative **2** which was then treated with the appropriate Grignard reagent to give the desired compounds **3a**,**b** in 60−70% yield. Route B therefore is a straightforward 4 step approach for the synthesis of the key intermediates **3a**,**b** in 42−49% overall yield. The significance of this approach is represented by the possibility of introducing two levels of functionalization: in the first level, different substituents can be introduced on the phenyl ring reacting the aldehyde **2** with different Grignard reagents while in the second level, the C-6 hydroxy group of the key intermediates can be additionally functionalized as shown in Scheme 5. Accordingly, compounds **3a**,**b** were oxidized with Dess−Martin periodinane affording the intermediates **13a**,**b** which were then alternatively converted into C-6 functionalized pyrimidinones or cytosino analogues (Scheme 5). Reaction of **13a**,**b** with methylmagnesium bromide afforded the alcohols **14a**,**b**, which were then converted into the C-6 vinyl derivatives **15a**,**b** after activation of the hydroxyl group as trifluoro acetate and subsequent acid-catalyzed elimination. On the other hand, selective C-4 chlorination of **13a**,**b** using $POCl_3$ and subsequent nucleophilic substitution with methanolic ammonia, gave the cytosino analogues **16a**,**b**. Reaction of the latter compounds with methylmagnesium bromide afforded **17a**,**b** which were dehydrated by refluxing with concentrated HCl to give the cytosine derivatives **18a**,**b**. It should be mentioned that the dehydration of compounds **14a**,**b** required the milder activation−elimination approach since reacting these compounds with refluxing HCl gave the S-demethylated product instead of the desired compounds **15a**,**b**. No traces of the S-demethylated side products were observed when the same reaction was carried out on cytosino analogues **17a**,**b**: the desired compounds **18a**,**b** were obtained in 20% yield together with easily removable decomposition products. The *S*-DABO analogues included in Scheme 5 were finally evaluated for cytotoxicity and anti-HIV-1-activity in comparison with nevirapine (NEV) and efavirenz (EFV), used as reference drugs (Table 1). The results of these assays allowed identification of an interesting lead compound (**16a**) possessing low cytotoxicity and submicromolar activity on MT-4 cells infected with both HIV-1 (wt) and clinically relevant mutants.

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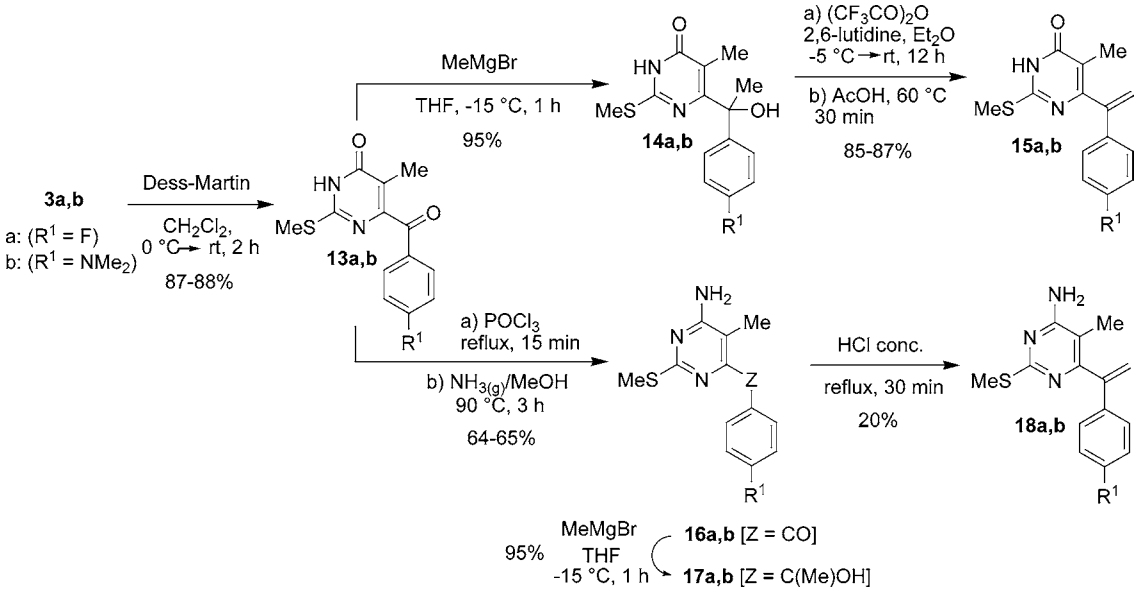
Scheme 5



In summary, a straightforward and versatile approach for the synthesis of new C-6 arylmethyl functionalized *S*-DABO analogues has been developed. An efficient 4 step procedure led us to the synthesis of the key intermediates **3a**,**b** (in 42−49% overall yield) while their further functionalization led to the identification of an interesting anti-HIV-1 lead compound (**16a**) belonging to a new family of *S*-DABO cytosine analogues. Exploitation of this procedure is underway in our laboratories for the synthesis of a large number of *S*-DABO cytosine analogues variously substituted on the C-6 arylmethyl moiety. An SAR study on this poorly explored class of derivatives might allow development of novel anti-HIV-1 inhibitors active on clinically relevant mutant strains.

Table 1. Cytotoxicity and Anti-HIV-1 Activity

	EC_{50} (μM)[a,b]				
compd	NL4-3 (wt)	K103N	Y181C	Y188L	CC_{50}[c]
16a	0.58	5.96	0.21	>90.25	90.25
NVP	0.08	1.8	0.87	5.6	>100
EFV	0.004	0.09	0.006	0.23	>0.3

[a] Data represent mean values of at least two experiments. [b] EC_{50}: effective concentration 50 or needed concentration to inhibit 50% HIV-induced cell death, evaluated with MTT method in MT-4 cells. [c] CC_{50}: cytotoxic concentration 50 or needed concentration to induce 50% death of noninfected cells evaluated with theMTT method in MT-4 cells.

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Supporting Information Available: Experimental procedures and spectral and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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