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Improved synthesis and preparative scale resolution of racemic monastrol

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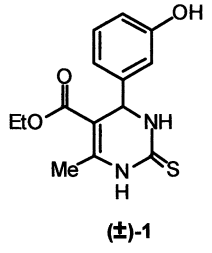
Abstract—The $Yb(OTf)_3$ catalyzed Biginelli cyclocondensation reaction of 3-hydroxybenzaldehyde, ethyl acetoacetate and thiourea afforded the corresponding dihydropyrimidine-2-thione, called monastrol, in 95% isolated yield. The chiral resolution of racemic monastrol, a mitosis blocker by kinesin Eg5 inhibition, was carried out on a preparative scale (ca. 100 mg) through diastereomeric *N*-3 ribofuranosyl amides.

The importance of the dihydropyrimidine (DHPM) ring as a pharmacophore is well established through the pharmacological activities of various derivatives as calcium channel antagonists (drugs candidates against cardiovascular diseases) and α_{1a} adrenergic receptor antagonists (drugs for the treatment of the benign prostatic hyperplasia).[1] DHPM derivatives are readily accessible products via the ketoester–aldehyde–urea (or thiourea) cyclocondensation reaction known as the Biginelli reaction.[2] The scope of this pharmacophore has been further increased by the identification of the 4-(3-hydroxyphenyl)-2-thione derivative **(±)-1** called monastrol (Fig. 1),[3] as a cell permeable lead compound for the development of new anticancer drugs. In fact, out of a 16 320-member collection of small molecules, monastrol **(±)-1** has been identified as a compound that specifically affects cell-division (mitosis) by a new mechanism which does not involve tobulin targeting. It has been established that the activity of **(±)-1** consists of the specific and reversible inhibition of the motility of the mitotic kinesin Eg5, a motor protein known to be required for spindle bipolarity. Given this biological activity, usable quantities of monastrol **(±)-1** in pure enantiomeric forms constitute a target of great importance. The only synthesis so far reported of **(±)-1** is that of Kappe and co-workers[4] by microwave-promoted condensation of ethyl acetoacetate, 3-hydroxybenzaldehyde, and thiourea in polyphosphate ester as reaction medium. The registered yield of isolated racemic **(±)-1** was ca. 60%. The separation of individual enantiomers was then carried out on a 0.02 mg scale by enantioselective HPLC and the absolute configuration established by CD spectroscopy. These results prompted us to report here both an improved synthesis of **(±)-1** in ca. 95% yield as well the chiral resolution of the racemate through diastereomeric *N*-3 glycosyl amides to give the individual *R*- and *S*-enantiomer on a preparative scale (typically 100 mg).

While in our recent work on the synthesis of glycosylated dihydropyrimidin-2-ones the three component system $CuCl/AcOH/BF_3Et_2O$ proved to be a quite efficient promoter of the Biginelli reaction,[5] the same catalyst turned out to be not compatible with the sulfurated version employing thiourea since the reaction between ethyl acetoacetate, 3-hydroxybenzaldehyde, and thiourea (3 equiv.) did not produce the desired product. Instead the use of $Yb(OTf)_3$ (0.1 equiv.) was quite beneficial since with this Lewis acid as a promoter the reaction afforded monastrol **(±)-1** (mp 184–186°C, lit.[4] 184–186) in 95% isolated yield by aqueous work-up[6] followed by flash chromatography (silica, cyclohexane/EtOAc 2:1) (Scheme 1). This result demonstrates the



(±)-1

Figure 1. The new mitosis blocker 4-(3-hydroxyphenyl)-3,4-dihydropyrimidine-2-thione, monastrol **(±)-1**.

Keywords: multicomponent reaction; Biginelli reaction; 3,4-dihydropyrimidine-2-thione; monastrol.

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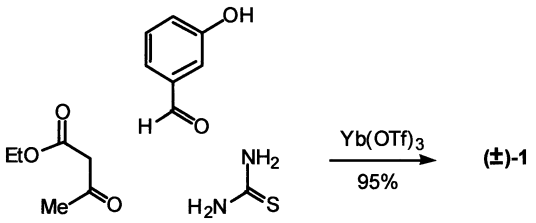


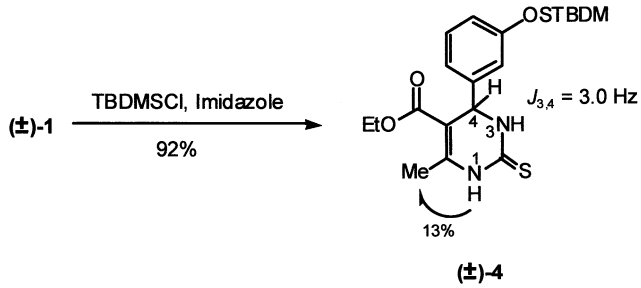
Scheme 1. *Conditions*: thiourea (3 equiv.), Lewis acid (0.1 equiv.), THF, reflux, 12 h.

compatibility of the lanthanide Lewis acid promoter $Yb(OTf)_3$ with the sulfurated version of the Biginelli reaction.[7]

Aiming at a chiral resolution of **(±)-1** on a preparative scale we first envisioned the separation of enantiomers via diastereomeric esters using the α-linked *C*-ribosyl carboxylic acid **2**.[8] The use of a sugar derivative as the chiral resolving agent followed our ongoing work on the synthesis of glycosylated monastrol analogues via Biginelli cyclocondensation reaction.[9] Under unoptimized conditions, esterification of the free hydroxy group of racemic monastrol **(±)-1** with **2** promoted by suitable condensation agents (Scheme 2) afforded the 1:1 mixture of the diastereomeric (4*R*)- and (4*S*)-3,4-dihydropyrimidine-2-thione derivatives **3** in 45% overall yield. Unfortunately, the separation of these diastereoisomers by column chromatography using a variety of solvents failed in our hands.

Another possible route for the resolution of **(±)-1** was via diastereomeric amides exploiting the selective *N*-acylation[2] of the NH group at the position 3 with respect to the position 1. However the protection of the phenolic hydroxy group was first required in order to avoid a competitive esterification. The selective *O*-silylation of **(±)-1** with *tert*-butyldimethylsilyl chloride was readily carried out by the standard method (Scheme 3) and the structure of the resulting *O*-TBDMS derivative **(±)-4**[10] (mp 146–148°C from cyclohexane/EtOAc) demonstrated by suitable NMR analysis. Accordingly a strong NOE was observed between H-1 and the adjacent Me at C-6 and a coupling constant $J_{3,4}=3.0$ Hz was measured.

Selective amide formation at N-3 of **(±)-4** with the β-linked *C*-glycosyl carboxylic acid **5**[11] was achieved via condensation with the acyl chloride **6** (3 equiv.) in toluene as a solvent (Scheme 4). The 1:1 mixture of diastereomeric (4*S*) and (4*R*) amides **7** was obtained in

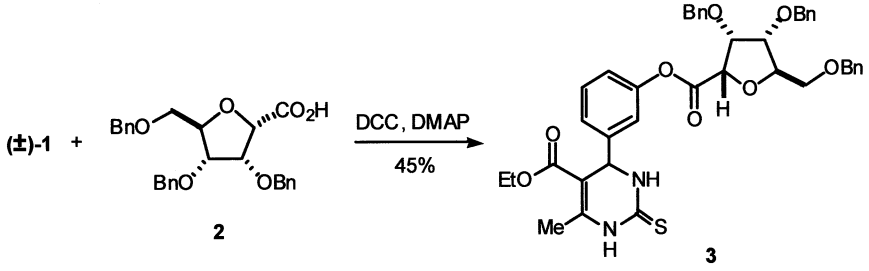


Scheme 3. *Conditions*: DMF, rt, 12 h.

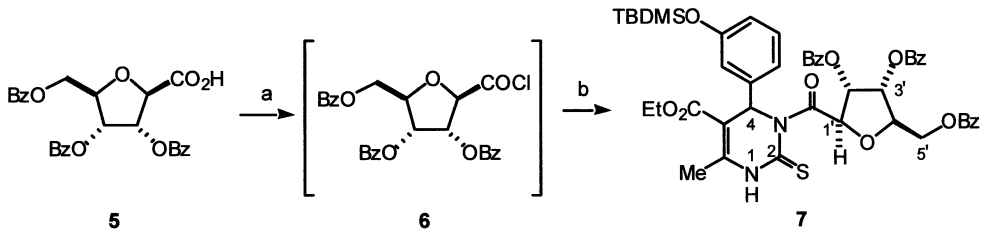
94% overall yield by flash chromatography (silica, cyclohexane/EtOAc 4:1) and the excess of the resolving agent **5** was almost quantitatively recovered. The selective *N*-acylation at the N-3 of **(±)-4** as well as the permanence of the silyl protective group on the oxygen atom of the phenyl ring were demonstrated by the absence of the coupling constant $J_{3,4}$ and the strong NOE between a methyl of the TBDMS group and an aromatic proton.

The two diastereomeric amides **7** were separated by flash chromatography (silica, toluene/diisopropyl ether 9:1 containing 0.3% of AcOH) starting from a typical scale of 400 mg mixture.[12] The first eluted was pure compound **(4*R*)-7**[10] ($[\alpha]_D$ −27, *c* 1.2, $CHCl_3$) in 40% yield[13] followed by the diastereoisomer **(4*S*)-7** (38% yield)[13] which however was impure by 15% of *O*-silylated monastrol **(±)-4**.[10] Chromatography (silica, cyclohexane/EtOAc 4:1) of the latter material afforded pure **(4*S*)-7**[10] ($[\alpha]_D$ 69, *c* 1.3, $CHCl_3$) in 36% yield.[13] The chiral sugar moiety and the *O*-TBDMS group were then removed in one step from each individual diastereoisomer **(4*R*)-7** and **(4*S*)-7** by treatment with EtONa in EtOH at room temperature overnight to give the two monastrol enantiomers **(4*R*)(−)-1** ($[\alpha]_D$ −71, *c* 1.6, MeOH; $[\alpha]_{436}$ −204, *c* 1.6, MeOH) and **(4*S*)(+)-1** ($[\alpha]_D$ 71, *c* 1.8, MeOH; $[\alpha]_{436}$ 204, *c* 1.8, MeOH, lit.[4] $[\alpha]_{436}$ 1.1, *c* 0.007, MeOH) in almost quantitative yield (95%) (Scheme 5). The assignment of the absolute configuration at C-4 of these compounds was confirmed by comparison of their CD spectra (Fig. 2) with those reported by Kappe.[4]

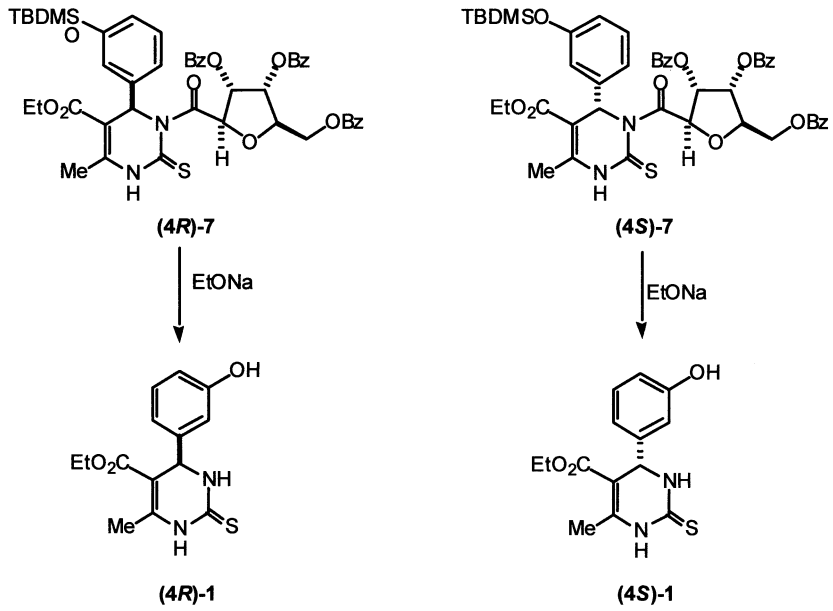
In order to prove that the basic removal of the chiral auxiliary acid occurred without loss of stereochemical integrity at C-4 of the DHPM ring, the enantiomers **(4*R*)(−)-1** and **(4*S*)(+)-1** were transformed into the corresponding diastereoisomeric *N*-glycosyl amides **(4*R*)-**



Scheme 2. *Conditions*: DMF, 120°C, 4 h.



Scheme 4. *Reagents and conditions*: (a) $SOCl_2$, DMF, toluene, 100°C, 2 h; (b) **(±)-4** (0.33 equiv.), toluene, 100°C, 4 h.



Scheme 5. *Conditions*: EtOH, rt, 12 h.

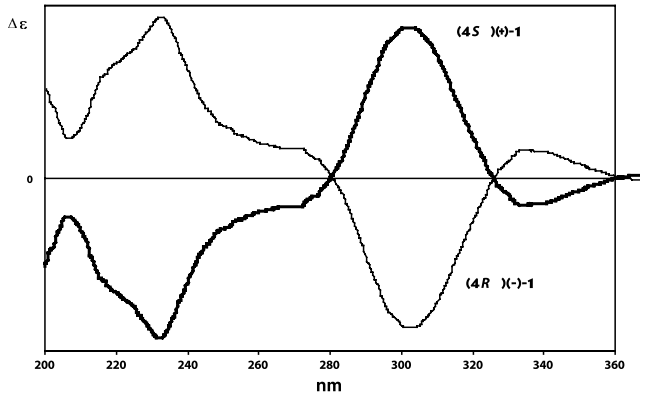


Figure 2. Experimental CD spectra of **(4*S*)(+)-1** and **(4*R*)(−)-1**.

7[10] and **(4*S*)-7**,[10] respectively, by protection of the hydroxy group and reaction with the acyl chloride **6**. Quite gratifyingly, each of these compounds appeared not to be contaminated by the other diastereoisomer by NMR analysis. Hence an enantiomeric purity ≥97–98% can be attributed to each monastrol isomer **(4*R*)-(−)-1** and **(4*S*)(+)-1**.

In conclusion an improved synthesis and a preparative scale resolution of monastrol **(±)-1** have been achieved, thus providing for the first time usable quantities of these isomers for biological studies.

Acknowledgements

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10. **(±)-4**: white solid; ^{1}H NMR (DMSO-d_6): $\delta = 10.18$ (s, 1H, H-1), 9.66 (bd, 1H, $J_{3,4} = 3.0$ Hz, H-3), 7.26–7.19 (m, 1H, Ph), 6.85–6.69 (m, 3H, Ph), 5.13 (bs, 1H, H-4), 4.02 (q, 2H, $J = 7.0$ Hz, OCH_2CH_3), 2.26 (s, 3H, CH_3), 1.11 (t, 3H, OCH_2CH_3), 0.95 (s, 9H, $C(CH_3)_3$), 0.19 (s, 6H, $Si(CH_3)_2$). **(4*R*)-7**: white foam; ^{1}H NMR (DMSO-d_6) selected data: $\delta = 11.78$ (s, 1H, H-1), 6.20 (d, 1H, $J_{1',2'} = 5.5$ Hz, H-1′), 6.11 (s, 1H, H-4), 5.89 (dd, 1H, $J_{2',3'} = 5.0$, $J_{3',4'} = 5.2$ Hz, H-3′), 5.78 (dd, 1H, H-2′), 4.79 (ddd, 1H, $J_{4',5'a} = 4.2$, $J_{4',5'b} = 4.8$ Hz, H-4′), 4.64 (dd, 1H, $J_{5'a,5'b} = 10.8$ Hz, H-5′a), 4.57 (dd, 1H, H-5′b), 2.00 (s, 3H, CH_3); MALDI-TOF MS: 879.0 (M^++H), 902.4 (M^++Na), 918.7 (M^++K). **(4*S*)-7**: white foam; ^{1}H NMR (DMSO-d_6) selected data: $\delta = 11.92$ (s, 1H, H-1), 6.26 (s, 1H, H-4), 6.14 (dd, 1H, $J_{1',2'} = 1.2$, $J_{2',3'} = 5.8$ Hz, H-2′), 6.01 (d, 1H, H-1′), 5.87 (dd, 1H, $J_{3',4'} = 7.5$ Hz, H-3′), 4.76 (ddd, 1H, $J_{4',5'a} = 4.5$, $J_{4',5'b} = 6.0$ Hz, H-4′), 4.65 (dd, 1H, $J_{5'a,5'b} = 10.8$ Hz, H-5′a), 4.55 (dd, 1H, H-5′b), 2.32 (s, 3H, CH_3); MALDI-TOF MS: 879.1 (M^++H), 902.6 (M^++Na), 918.6 (M^++K).
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13. Yield calculated with respect to the initial amount of *O*-TBDMS derivative **(±)-4** subjected to the chiral resolution.