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Efficient Synthesis of the Plant Growth Regulator Ancymidol

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التصنيع الفعال للأنسيميدول المتحكم في نمو النبات

هايكو باتزلت

خلاصة: مانع سايتوكروم بـ ـ450 أنسيميدول يستخدم كمبطأ لنمو النبات وله تطبيقات طبية أخرى. ولكن سعره المرتفع يضع قيوداً إقتصادية لتطبيقاته في مجالات واسعة. في هذا البحث، تم إيجاد عملية تصنيع قصيرة ومنتجة للأنسيميدول بكميات كافية وبتكلفة أقل.

ABSTRACT: The important cytochrome P450 inhibitor ancymidol is used as a plant growth retardant and has potential for various medicinal applications. However its high price sets economic limits to large-scale applications. Here a short and high-yielding synthesis is reported, providing ancymidol in substantial amounts in a cost- and time-efficient way.

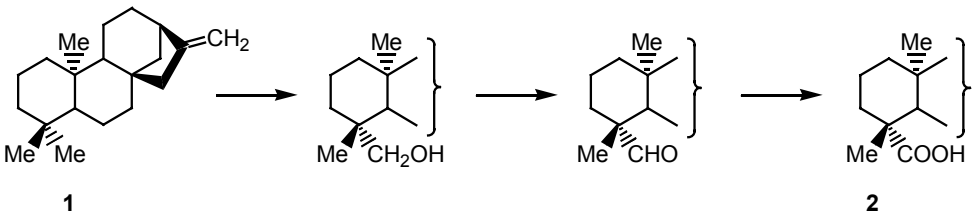
KEYWORDS: Plant Growth Regulator; Cytochrome P450; Oxygenase Inhibitor.

1. Introduction

Plant growth retardants are applied to agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering plant productivity. Most growth retardants act by inhibiting gibberellin biosynthesis. To date, four different types of such inhibitors are known: (a) Onium compounds, such as chlormequat chloride, mepiquat chloride, chlorphonium, and AMO-1618, which block the cyclases copalyl-diphosphate synthase and ent-kaurene synthase involved in the early steps of gibberellin metabolism; (b) Structural mimics of 2-oxoglutaric acid, which is the co-substrate of dioxygenases that catalyze late steps of gibberellin formation. Acylcyclohexanediones, e.g. prohexadione-Ca and trinexapac-ethyl and daminozide, block particularly 3 beta-hydroxylation, thereby inhibiting the formation of highly active gibberellins from inactive precursors; (c) 16,17-Dihydro-GA(5) and related structures act most likely by mimicking the GA precursor substrate of the same dioxygenases; (d) Compounds with an *N*-containing heterocycle, e.g. ancymidol, flurprimidol, tetcyclacis, paclobutrazol, uniconazole-P, and inabenfide (Rademacher, 2000). The crucial molecular effect of these pyrimidine and triazole derivatives seems to be the inhibition of gibberellins biosynthesis, specifically at the oxidation of ent-kaurene (**1**) to ent-kaurenoic acid (**2**, scheme 1) (Coolbaugh *et al.*, 1978). The transformation involves three oxidative steps, which all require NADPH and molecular oxygen, and are catalyzed by cytochrome P450 mono-oxygenases (Murphy and West, 1978). All three steps are inhibited by ancymidol with comparable efficiency (Coulson *et al.*, 1984).

Furthermore, ancymidol was found to inhibit certain oxidations on steroid backbones, which led to extensive pharmaceutical testing of the molecule. On the basis of this lead structure, for example, researchers from the Eli Lilly laboratories tried to develop inhibitors for the aromatase of human estrogen biosynthesis with the goal of providing novel breast cancer therapeutics. Pyrimidine derivatives show a higher activity than their (partially) saturated analogues, and the

hydroxyl and cyclopropyl moieties seem to be equally important for most observed effects (Davenport *et al.*, 1968; Taylor *et al.*, 1987). Cyclopropyl derivatives have repeatedly been described as suicide substrates for eukaryotic cytochrome P450s (Testa and Jenner, 1981; Ortiz-De-Montellano, 1988; Ortiz-De-Montellano and Reich, 1986) or were used for biosynthesis investigations (Patzelt and Robinson, 1993).



Scheme 1. The oxidation of *ent*-kaurene (**1**) to *ent*-kaurenoic acid (**2**) in *Echinocystis macrocarpa* (Cucurbitaceae).

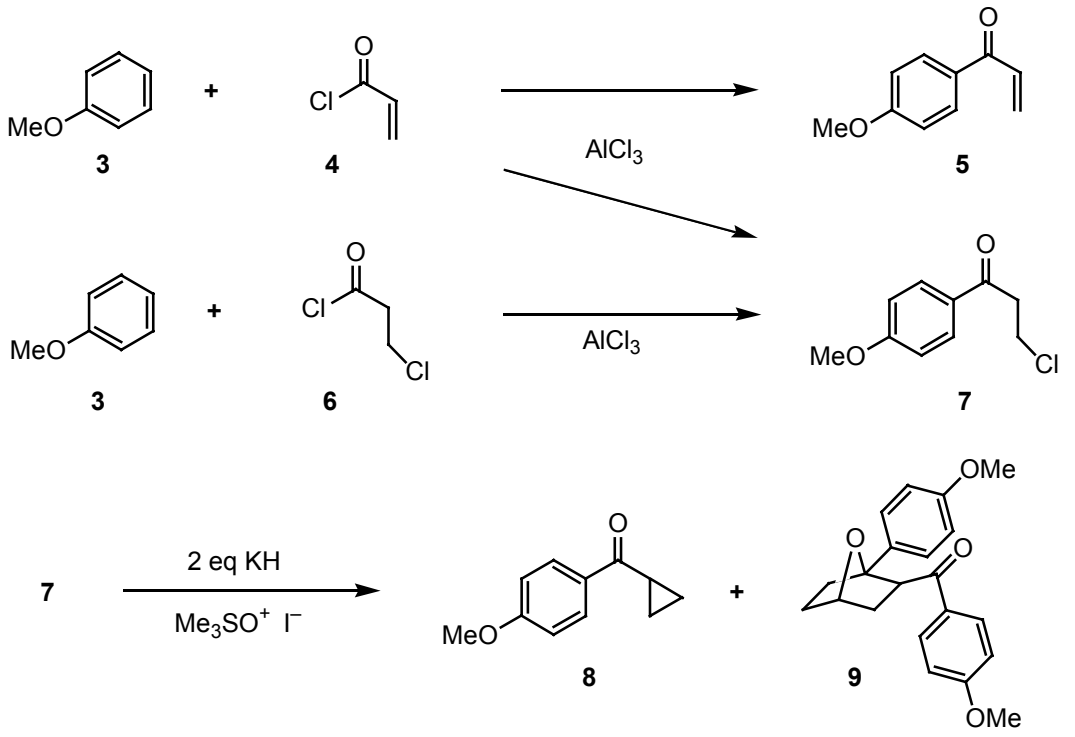
2. Synthesis of the P450 inhibitor ancymidol

Although ancymidol has recently become commercially available, the high price makes large-scale applications a costly enterprise. The single published, industrial, synthetic approach, on the other hand, does not provide satisfying experimental details (Taylor *et al.*, 1987). It appeared reasonable to establish a short synthesis on the basis of the arylation of *p*-anisyl cyclopropyl ketone (**8**).

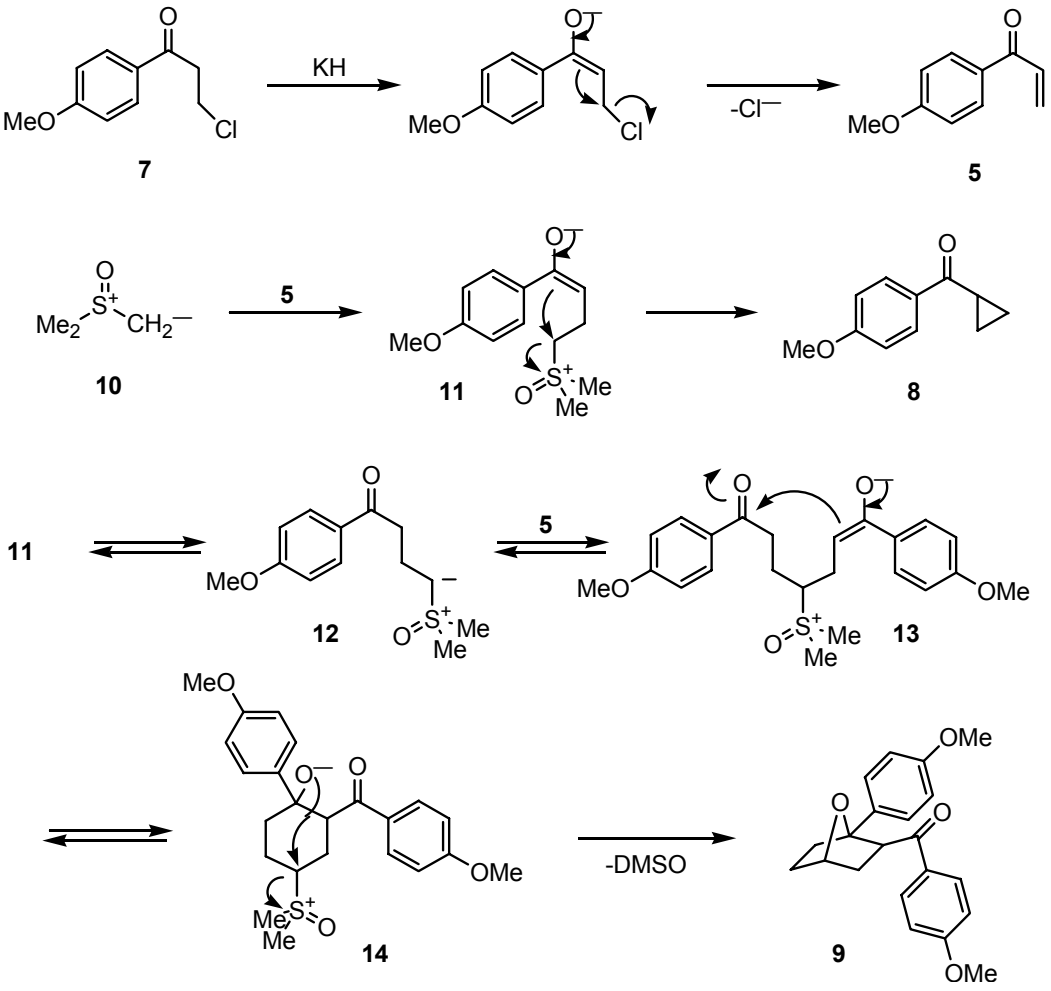
The Friedel-Crafts acylation of anisole (**3**) with acryloyl chloride (**4**) gave the expected *p*-anisyl vinyl ketone (**5**) in only mediocre yields. Apart from polymerisation products, up to 40% of the formal hydrochlorination product **7** were isolated. This chloride **7**, however, was available directly from anisole and 3-chloropropanoyl chloride (**6**) in more than 90% yield. Neither base-catalyzed nor thermal (Santelli and Bertrand, 1973) elimination of HCl from **7** would produce **5** in satisfactory yields, but when **7** was directly treated with trimethyloxosulfonium iodide (Corey and Chaykovsky, 1965) and two equivalents of potassium hydride, the desired *p*-anisyl cyclopropyl ketone (**8**) was obtained in up to 80% yield (scheme 2). A proposed one-pot formation of **8** by the reaction of **3** with 4-chlorobutanoyl chloride (Close, 1957) did not give synthetically useful results.

When the volume of solvent (DMSO) in the transformation of **7** into **8** was reduced, a by-product, which could always be detected on tlc in the reaction mixture, became the main product. It could be purified and characterized, and the unexpected bicyclic structure **9** was assigned. It presumably arises from two molecules of the ketone **7** and one methyl group from the oxosulfonium salt (scheme 3).

For the formation of the cyclopropyl moiety, an *in situ* elimination of HCl from **7** can be envisaged. The double bond of the resulting unsaturated ketone **5** would then undergo a Michael-type attack by dimethyloxosulfonium methylide **10**, derived from trimethyloxosulfonium iodide by deprotonation. Ring closure can occur via an intramolecular substitution of the oxosulfonium group in **11** by the enolate carbon (Trost and Melvin, 1975; Block, 1981). When the concentration of **5** and trimethyloxosulfonium iodide in the solution becomes too high, the enolate in **11** might be protonated before the cyclization takes place. After deprotonation at the very acidic sulfonium bearing carbon atom C(4), **12** can react with a second molecule of **5** or **7**. An intramolecular aldol reaction would reversibly form the six-membered ring of **14**, which, as soon as the oxide is in 1,4-trans position to the sulfonium group, can cyclize to give **9** (scheme 3). This rationalization, of course, is so far purely speculative.



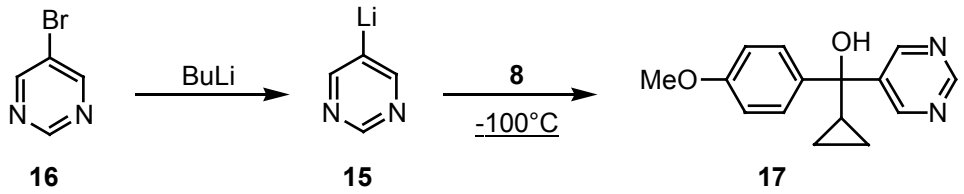
Scheme 2. Synthesis of *p*-anisyl cyclopropyl ketone (**8**).



Scheme 3. Proposed formation of the bicyclic side product **9** during the ancymidol synthesis.

The concluding nucleophilic addition of 5-lithiopyrimidine (**15**) onto the carbonyl group of **8** proceeded cleanly as long as the temperature was kept below -100°C. It was possible to generate **15** *in situ* by the addition of butyl lithium to a solution of freshly sublimed 5-bromopyrimidine (**16**) and **8**. Ancymidol (**17**) was obtained in 73% yield after recrystallization (scheme 4). It should be mentioned that even minor warming of the reaction mixture or minute impurities in the starting material led to considerable reductions in the yield.

Ancymidol can thus be prepared in a very straight-forward and efficient synthesis in 55% overall yield from inexpensive anisole, and is now available in multigram quantities for biological experiments.



Scheme 4. Transformation of *p*-anisyl cyclopropyl ketone (**8**) to ancymidol (**17**).

3. Experimental

3.1 General

All solvents and reagents were purchased from Aldrich or Fluka in their highest available quality. Solvents were distilled before use and dried over an appropriate desiccant (Perrin and Armarego, 1988). Reactions under anhydrous conditions were performed in a N_2-atmosphere using standard Schlenck techniques (Casey *et al.*, 1990).

3.2 3-Chloro-1-(4-methoxyphenyl)-1-propanone (7)

Anisole (**3**; 54.0 g, 480 mmol) and $AlCl_3$ (64.0 g, 480 mmol) were stirred in dichoromethane (240 ml), at 0°C under an N_2-atmosphere, until the solution became clear and homogenous (30 min). The flask was kept in the ice bath and 3-chloropropanoyl chloride (**6**; 52.0 g, 400 mmol) was added dropwise at such a speed that the solvent mildly boiled. The colour of the solution changed from bright yellow to dark red. The ice bath was removed and the reaction was stirred for further 2h at room temperature before being poured onto crushed ice. Extraction with hexane and evaporation of the dried ($MgSO_4$) solvent gave **7** (74.2 g, 370 mmol, 92.5%) as light rose-coloured crystals, which could be recrystallized from hexane. m.p.: 62-63°C. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.90 (*d*, 2H, [C(2',6')H], $^3J_{ortho}$ = 8.9 Hz); 6.95 (*d*, 2H, [C(3',5')H], $^3J_{ortho}$ = 8.9 Hz); 3.96 (*s*, 3H, [OCH_3]); 3.89 (*t*, 2H, [C(2)H], ^3J =6.9 Hz); 3.45 (*t*, 2H, [C(3)H], ^3J = 6.9 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$): 195.1 [C(1)]; 163.8 [C(4')]; 130.3 [C(2',6')]; 129.5 [C(1')]; 113.8 [C(3',5')]; 55.5 [OCH_3]; 40.8 [C(2)]; 38.9 [C(3)]. IR (KBr): 3010*w* (νCH aromatic), 2950*m* and 2920*w* (νCH aliphatic), 2840*m* (νCH in CH_3), 1670*s* (νC=O), 1595*s* and 1510*s* (νCC aromatic), 1455*m*, 1435*m*, 1415*m*, 1350*s*, 1305*m*, 1260*s* (νCO), 1205*m*, 1170*s*, 1110*m*, 1070*w*, 1025*m*, 985*m*, 835*s* and 780*s* (δCH out-of-plane), 685*m* (νCCl). MS (ci, NH_3): 218.2 (29.9%), 216.1 (100%, $[M+NH_4]^+$), 201.2 (5.1%), 200.2 (6.4%), 199.0 (22.8%, $[M+H]^+$). MA for $C_{10}H_{11}ClO_2$ (198.65): calc. C 60.46, H 5.58, Cl 17.85; found C 60.39, H 5.71, Cl 17.63.

3.3 Trimethyloxosulfonium iodide

A solution of methyl iodide (45 ml, 68 g, 480 mmol) in anhydrous DMSO (16 ml, 24 g, 310 mmol (dist. from CaH_2)) was refluxed for 3 d under an N_2-atmosphere. The solution darkened and a solid precipitated. After cooling to room temperature the solution was filtered, and the solid was

washed with small portions of $CHCl_3$ and was dried *in vacuo* to give white crystals (16.4 g, 74.5 mmol, 24%). m.p.: 192-195°C. ^{1}H-NMR (300 MHz, D_2O): 3.24 (*s*). ^{13}C-NMR (50 MHz, D_2O): 64.2. IR (KBr): 2960*s* and 2870*s* (νCH), 1400*s* (δCH), 1335*w*, 1310*m*, 1225*s* (νCS), 1035*s* (νS=O), 950*s*, 750*m*. MS (ci, NH_3): 93.2 (100%, $[(CH_3)_3SO]^+$). MA for C_3H_9JOS (220.07): calc. C 16.38, H 4.10; found C 16.50, H 4.32.

3.4 Cyclopropyl-(4-methoxyphenyl)-methanone (8)

Anhydrous DMSO (350 ml, dist. from CaH_2) was slowly dropped onto a solid, intensively stirred mixture of ground trimethyloxosulfonium iodide (54.0 g, 240 mmol) and NaH (10.5 g, 480 mmol (washed with anhydrous hexane and dried *in vacuo*)), at 0°C under an N_2-atmosphere. The resulting slurry was stirred for further 30 min at room temperature. It was then cooled to 10°C inside temperature before 3-chloro-1-(4-methoxyphenyl)-1-propanone (**7**; 37.2 g, 240 mmol) in anhydrous DMSO (120 ml) was added. The mixture was kept at that temperature for another 5 min and was then stirred at room temperature for further 2 h. The reaction was poured onto ice and was extracted with DCM (3x). The combined organic phases were washed twice with H_2O and brine, and were dried over $MgSO_4$. Evaporation of the solvent and column chromatography (20% EtOAc in hexane) gave the cyclopropylketone **8** (34.3 g, 195 mmol, 81%) as a slightly yellow oil. ^{1}H-NMR (300 MHz, CDC_3): 7.80 (*d*, 2H, [C(2',6')H], $^3J_{ortho}$ = 8.9 Hz); 6.85 (*d*, 2H, [C(3',5')H], $^3J_{ortho}$ = 8.9 Hz); 3.76 (*s*, 3H, [OCH_3]); 2.45 (*m*, 1H, [C(2)H]); 1.05 (*m*, 2H) and 0.8 (*m*, 2H) [C(3,4)H]. ^{13}C-NMR (50 MHz, $CDCl_3$): 199.0 [C(1)]; 163.2 [C(4')]; 131.0 [C(1')]; 130.2 [C(2',6')]; 113.6 [C(3',5')]; 55.4 [OCH_3]; 16.6 [C(2)]; 11.1 [C(3,4)]. IR ($CHCl_3$): 3000*w* (νCH aromatic), 2980*m* and 2930*w* (νCH aliphatic), 2840*m* (νCH in CH_3), 1660*s* (νC=O), 1600*s*, 1575*m*, and 1510*s* (νCC aromatic), 1460*m*, 1420*m*, 1385*s*, 1305*m*, 1260*s* (νCO), 1235*m*, 1195*w*, 1170*s*, 1120*w*, 1030*s*, 1010*w*, 990*s*, 835*s*. MS (ci, NH_3): 177.3 (100%, $[M+H]^+$). $C_{11}H_{12}O_2$ (176.22).

3.5 2-(4-Methoxyphenacyl)-1-(4-methoxyphenyl)-7-oxabicyclo[2.2.1]heptane (9)

Anhydrous DMSO (50 ml; dist. from CaH_2) was slowly dropped onto a solid, intensively stirred mixture of ground trimethyloxosulfonium iodide (17.6 g, 80 mmol) and NaH (3.5 g, 160 mmol; washed with anhydrous hexane and dried *in vacuo*), at 0°C under an N_2-atmosphere. The resulting slurry was stirred for further 30 min at room temperature. It was then cooled to 10°C inside temperature before 3-chloro-1-(4-methoxyphenyl)-1-propanone (**7**, 12.4 g, 80 mmol) in anhydrous DMSO (30 ml) was added. The mixture was kept at that temperature for another 5 min and was then stirred at room temperature for further 2 h. The reaction was poured onto ice and was extracted with DCM (3x). The combined organic phases were washed twice with H_2O and brine, and were dried over $MgSO_4$. Evaporation of the solvent gave a yellow oil which solidified upon cooling to 0°C. The precipitate was filtered off, washed with cooled anhydrous ether (the filtrate contained cyclopropyl-ketone **8**) and recrystallized from ether/hexane (1:1) to give the bicyclic ketone **9** (5.4 g, 30.9 mmol, 38.6%) in the form of white powdery crystals. m.p.: 152-153°C. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.95 (*d*, 2H, [C(2'',6'')H], $^3J_{ortho}$ = 8.9 Hz); 7.30 (*d*, 2H, [C(2''',6''')H], $^3J_{ortho}$ = 8.9 Hz); 6.95 (*d*, 2H, [C(3'',5'')H], $^3J_{ortho}$ = 8.9 Hz); 6.85 (*d*, 2H, [C(3''',5''')H], $^3J_{ortho}$ = 8.9 Hz); 3.85 (*s*, 3H, [OCH_3]); 3.80 (*s*, 3H, [OCH_3]); 3.30 (*m*, 1H, [C(4)H]); 3.22 (*d*, 1H, [C(2)H], ^3J = 1.0 Hz); 2.31 (*m*, 4H, [C(3,6)H]); 1.75 (*m*, 2H, [C(5)H]). ^{13}C-NMR (50 MHz, $CDCl_3$): 200.4 [CO]; 163.4 [C(4'')]; 159.0 [C(4''')]; 133.3 [C(1'')]; 130.5 [C(2'',6'')]; 129.0 [C(1''')]; 126.5 [C(2''',6''')]; 113.8 [C(3''',5''')]; 113.7 [C(3'',5'')]; 60.6 [C(4)]; 59.6 [C(1)]; 55.5 [OCH_3]; 55.3 [OCH_3]; 40.2 [C(2)]; 28.6, 26.9, and 23.2 [C(3,5,6)]. IR (KBr): 3060*w* (νCH aromatic), 2995*m*, 2960*s*, 2940*s*, and 2910*s* (νCH aliphatic), 2840*m* (νCH in CH_3), 1655*s* (νC=O), 1600*s*, 1570*s* and 1510*s* (νCC aromatic), 1455*m*, 1440*m*, 1420*m*, 1370*m*, 1355*w*, 1315*s*, 1285*w*, 1250*s* (νCO), 1200*m*, 1180*s*, 1110*m*, 1030*s*, 990*m*, 960*m*, 895*w*, 845*m*, 825*s* (δCH out-of-plane), 790*w*, 765*m* (δCH out-of-plane),

690*w*, 630*w*, 610*m*. MS (ci, NH_3): 340.2 (15.5%), 339.0 (100%, $[M+H]^+$), 322.3 (4.7%), 321.1 (32.1%, $[M+H-H_2O]^+$). MA for $C_{21}H_{22}O_4$ (338.40): calc. C 74.46, H 6.72; found C 74.03, H 6.72.

3.6 Cyclopropyl-(4-methoxyphenyl)-(5-pyrimidinyl)-methanol (ancymidol) (17)

n-Butyllithium (1.6 M in hexane; 31.3 ml, 50.0 mmol) was slowly added (over 2 h) to a solution of 5-bromopyrimidine (**16**, 8.0 g, 50.0 mmol; sublimed *in vacuo*) and the anisyl-cyclopropyl-ketone **8** (8.8 g, 49.9 mmol) in anhydrous THF (100 ml), at -100°C under a N_2-atmosphere. Stirring at that temperature was continued for a further 1 h before the solution was poured into NH_4Cl and extracted with EtOAc. The combined organic layers were washed with NH_4Cl, $NaHCO_3$, and brine, and dried over $MgSO_4$. Evaporation of the solvent gave a slightly yellow oil (12.4 g), which crystallized over night in the refrigerator. Re-crystallisation from EtOAc gave pure ancymidol (**17**, 9.4 g, 36.7 mmol, 73.5%) as a white powder. m.p.: 110°C. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.00 (*s*, 1H, [C(2''')H]); 8.69 (*s*, 2H, [C(4''',6''')H]); 7.38 (*d*, 2H, [C(2'',6'')H], $^3J_{ortho}$ = 8.9 Hz), 6.88 (*d*, 2H, [C(3'',5'')H], $^3J_{ortho}$ = 8.9 Hz); 3.81 (*s*, 3H, [OCH_3]); 2.79 (*s*, 1H, [72]); 1.55 (*m*, 1H, [C(1')H]); 0.75 (*m*, 1H, [C(2')H]); 0.53 (*m*, 3H, [C(2',3')H]). ^{13}C-NMR (50 MHz, $CDCl_3$): 159.2 [C(4'')]; 156.9 [C(2''')]; 155.1 [C(4''',6''')]; 140.7 [C(1''')]; 137.3 [C(1'')]; 128.3 [C(2'',6'')]; 113.8 [C(3'',5'')]; 74.4 [C(1)]; 55.3 [OCH_3]; 21.5 [C(1')]; 2.5 and 1.0 [C(2',3')]. IR (KBr): 3200*br s* (νOH), 3000*m* (νCH aromatic), 2970*w* and 2930*w* (νCH aliphatic), 2830*w* (νCH in CH_3), 1620*s*, 1580*s*, 1560*s*, and 1510*s* (νCC aromatic), 1460*m*, 1435*m*, 1435*s*, 1400*m*, 1305*m*, 1250*s* (νCO), 1205*m*, 1170*m*, 1145*m*, 1110*w*, 1100*w*, 1050*w*, 1030*m*, 1010*m*, 990*m*, 980*m*, 960*w*, 910*w*, 880*m*, 850*w*, 825*s* (δCH out-of-plane), 805*w*, 790*w*, 780*w*, 720*m* (δCH out-of-plane), 635*m*. MS (ci, NH_3): 257.3 (100%, $[M+1]^+$), 228.3 (40%). MA for $C_{15}H_{16}N_2O_2$ (256.31): calc. C 70.29, H 6.29, N 10.93; found C 70.43, H 6.39, N 10.72.

4. Acknowledgements

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