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Process Research and Development for the Production of Intermediates for the Synthesis of Carbocyclic Nucleosides

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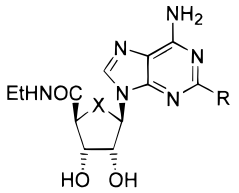
Abstract:

The synthesis of [3a*R*,4*S*,6*R*,6a*S*]-6-amino-*N*-ethyltetrahydro-2,2-dimethyl-4*H*-cyclopenta-1,3-dioxole-4-carboxamide, a key single enantiomer intermediate to carbocyclic nucleosides such as adenosine agonists, is reported involving a scalable catalytic osmium tetraoxide dihydroxylation of (−)-2-azabicyclo[2.2.1]-hept-5-en-3-one. The acetonide-protected diol [3a*S*,4*R*,7*S*,7a*R*]-tetrahydro-2,2-dimethyl-4,7-methano-1,3-dioxolo[4,5-*c*]pyridin-6(3a*H*)-one is subject to lactam ring opening with anhydrous ethylamine to give the carbocyclic key intermediate. The rate of this known reaction, carried out in a pressure vessel, is considerably enhanced by acid catalysis using ethylammonium ion. In addition, the need for a pressure vessel is circumvented by using anhydrous ethylamine with acid catalysis. Alternatively stoichiometric ethylammonium ion in an appropriate cosolvent can be used to form the key intermediate in high yield.

Introduction

As a result of the inherent instability of natural nucleosides to the action of nucleoside phosphorylases and hydrolases,[1] considerable research into the therapeutic effects of carbocyclic analogues of purine and pyrimidine nucleosides[2] has been carried out in order to develop more stable analogues with greater activity and duration of action. As an example, adenosine is a potent coronary vasodilator but is short-lived and orally inactive.[3] These shortcomings initiated attempts to develop orally active derivatives with higher potency.[3] Daly[4] reported that 5′-deoxy-5′-(ethylamino)-5′-oxoadenosine (**1**) exhibits potent activity on the adenosine A_2 receptor which is associated with vasodilation and hypertension in peripheral blood vessels. Chen[5] was interested in developing novel carbocyclic derivatives of **1** that would have increased bioavailability and thus combined the *N*-ethylcarboxamide feature of **1**, carbocyclic replacement of the ribose unit, and C2 substitution on the purine heterocycle in an attempt to produce more potent derivatives **2**. The overall synthesis (Scheme 1) started from racemic 2-azabicyclo[2.2.1]hept-5-en-3-one (**3**). This was converted to the lactam diol **4** *via* an osmium tetraoxide catalysed dihydroxylation.[2] The stages from **4** involved acetonide formation followed by lactam ring opening of **5** with anhydrous ethylamine in a steel pressure vessel.[5] The overall yield from **3** to the amide **6** was 76% (80% yield for **3** to **4**[2] and 95% yield for **4** to **6**[5]). Resolution of the racemic ethylamide **6** was achieved using dibenzoyl-L-tartaric acid to form **7** with the correct stereochemistry for carbocyclic nucleosides such as **2**.



1 R = H , X = O
2 R = R^1R^2N , X = CH_2
R^1 = various alkyl, aryl[5]
R^2 = H, Me

It was anticipated that a scalable process to **7** (Scheme 2) starting from optically pure (−)-**3** (compound **8**) could be accomplished with suitable process development. The biocatalytic resolution of **3** with lactamases is a highly effective process that has provided tonne quantities of the single (−)-enantiomer. This is the subject of a separate paper.[6] We herein report the procedures used at pilot plant scale for the manufacture of **7** involving a key catalytic osmium tetraoxide dihydroxylation of **8** and ethylamine ring opening of **10**. The development phase of the oxidation presented considerable problems associated with catalyst inhibition which needed obvious control prior to scale-up. Endeavours to rationalise this problem common to many catalytic dihydroxylation reactions have been made. At Chiroscience we had a demand for the single enantiomer ethylamide **7** as a stable derivative or salt since the free base **7** is a viscous hygroscopic oil. The benzoic acid salt **11** of amine **7** was found to be a highly crystalline solid easily purified by recrystallisation. We embarked upon process research and development which eventually resulted in the manufacture of multikilogram quantities of this key intermediate **11**. A particular area of process discovery was the circumvention of the scalable but facility-limiting and costly amidation that had required use of a pressure reactor.

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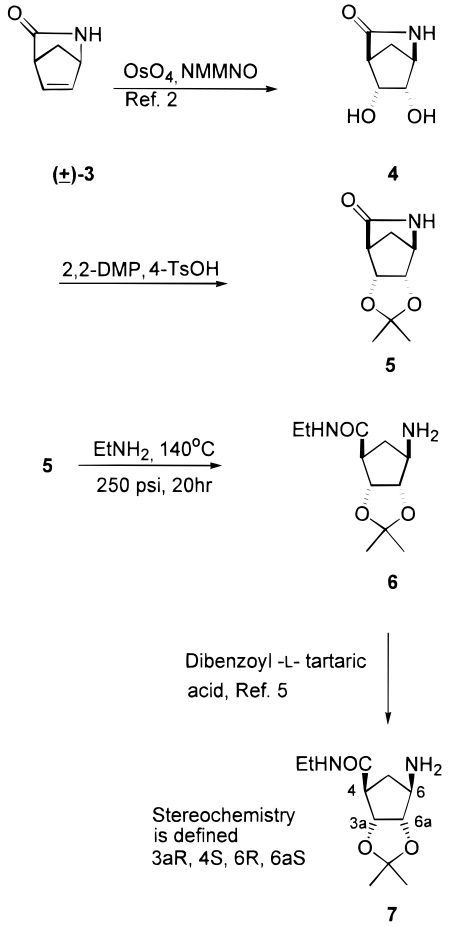
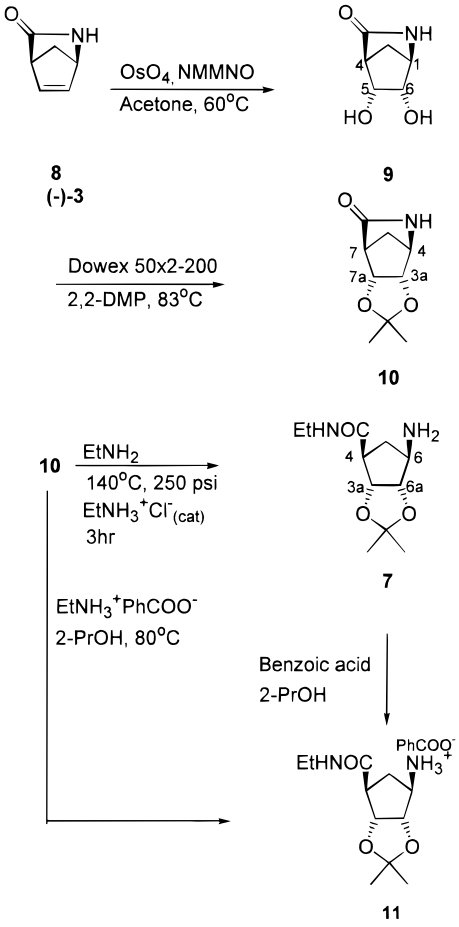
Scheme 1



Scheme 2



Results and Discussion

When process research and development to **7** had commenced, it was necessary to consider the potential for formation of unwanted diastereomers. The osmium tetraoxide *cis* dihydroxylation of **8** is *exo* selective by virtue of the constraints of the bicyclic framework.[7] Interestingly monocyclic cyclopentenecarboxylates are similarly dihydroxylated where stereofacial selectivity results from the presence of the carboxamide functionality.[8] The stereofacial selectivity for the transformation of **8** to **9** was ascertained by de determination. Thus, following dihydroxylation of **8** in >99.5% ee[9] and de,[10] all four stereogenic centres were clearly defined.

***Cis* Dihydroxylation of (−)-Lactam (8).** In initial studies catalytic osmium tetraoxide (0.23 mol %) was added to a solution of **8** in 50% *tert*-butyl alcohol in water containing *N*-methylmorpholine *N*-oxide (NMO) at 50 °C. After an initiation period of approximately 2−4 min, a steady rise in temperature to 100 °C ensued, with reaction complete within 0.5 h. Due to the high solubility of the diol in water, the workup proved awkward. Simple extractive processing with dichloromethane was not particularly successful due to the high water solubility of **9**. Extraction with the more polar solvent *n*-butyl alcohol worked extremely well. Concentration of the extracts facilitated partial crystallisation of the product, which could be triturated with acetone and collected by filtration. For initial kilogram requirements the above procedure served well to produce suitably pure **9**. However, the uncontrollable exothermicity associated with this practice of adding the catalyst last necessitated process development to find an alternative procedure for the pilot plant chemistry. It must also be mentioned that in early development work this mode of addition had been chosen deliberately since a reversal of the catalyst and NMO additions resulted in catalyst inhibition at 70−80% reaction. The presence of a dark red/brown coloration rather than the black/brown colour typical of the transient Os(VI) species expected became evident when catalyst inhibition occurred. However, conditions were developed under which catalyst could be added first.

As the basis of a more suitable procedure, the choice of acetone as a reaction solvent worked very well. The amount of water present was reduced considerably, being only that from the 50% w/w aqueous NMO, with the benefit of allowing easier product recovery. The main process change was to add catalytic osmium tetraoxide to an acetone solution of olefin **8** under conditions of gentle reflux. The steady

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(9) We ascertained the de/ee by GC on the *N*-benzoylamides obtained by dehydrative coupling of **7** and diastereomers to benzoic acid. Thus treatment of compound **11** with DCC or the equivalent gave the *N*-benzoylamide of **7** readily analysed using a Hewlett Packard 5890 GC fitted with a 30 m HP5 capillary column under 15 psi of helium; a temperature gradient was used: 200 °C for 10 min, to 300 °C at 10 °C/min, remaining at 300 °C for 10 min. Injection temperature: 250 °C. Detection was achieved using a Hewlett Packard mass selective detector (range: 50−400). Detector temperature: 280 °C.
(10) Traces of *endo* diol were present in the recrystallisation liquors from a pilot plant campaign. A sample of *N*-benzoylamide was obtained for de analysis.[9]

addition of NMO over 2−3 h allowed osmium(VI) glycolate hydrolysis and regeneration of Os(VIII). With this mode of addition, conversions in excess of 95% were achieved, although if NMO addition was allowed to slow or temperature allowed to drop, then catalytic inhibition would occur. An efficient workup was developed involving concentration of the reaction liquor to minimum stir volume in the pilot plant vessel followed by crystallisation of compound **9** from methanol.

It is postulated that inhibition of catalysis is due to the formation of a stable osmium(VI) diglycolate by analogy with other work reported[11] and it is this species which gives rise to the dark red/brown coloration observed in the dihydroxylation of **8**. It is suggested that, when there is a deficiency of NMO oxidant, the substrate−osmate (monoglycolate) complex diverts to a catalytic cycle involving this osmium(VI) diglycolate which ultimately leads to the loss of catalyst. It is noteworthy that further catalyst addition did not give the expected turnover but instead rapid catalyst inhibition occurred. This problem remains curious although we now have conditions under which reproducible >90% conversion is achieved.

Acetonide Protection of the *cis*-Diol 9, Ring Opening of Lactam Acetonide 10 with Ethylamine, and Benzoic Acid Salt Formation (11): 1 kg Scale. In early studies acetonide **10** was prepared from diol **9** using typical conditions of reflux with 2,2-dimethoxypropane and catalytic 4-toluenesulphonic acid (TsOH). Complete dissolution invariably indicated complete reaction. Upon cooling of the blackened solution the acetonide **10** crystallised to return a 50−60% yield. Further crops (20−30%) were obtained by concentrating the mother liquor and crystallising subsequent crops from the residual liquor or by addition of acetone. One problem with the above method was that eliminating trace TsOH from acetonide **10** and subsequently final product **11** proved difficult. However, in the case of the former this proved to be fortuitous since this catalytic amount of TsOH present was found to have considerable effects upon the rate of lactam ring opening of the acetonide **10** with ethylamine to form amine **7**. Early studies in this transformation involved using the reported conditions[5] whereby **10** is dissolved in anhydrous ethylamine in a pressure vessel and the solution heated to 140 °C, 250 psi for 15−20 h. In our hands the reaction time for complete conversion was found to be quite variable with reaction times between 7 and 20 h for 80−90% conversion. The initial requirement of 1 kg of **7** was achieved *via* repetitive chemistry using a 600 mL Parr reactor. The benzoic acid salt **11** of **7** was prepared straightforwardly by addition of a propan-2-ol solution of benzoic acid to **7** predissolved in the same solvent. Final product **11** could be recrystallised as necessary from propan-2-ol.

Acetonide protection of the *cis*-Diol 9 and Ring Opening of Lactam Acetonide 10 with Ethylamine: Pilot Plant Scale. More appropriate conditions for the production of acetonide **10** were established by using catalytic Dowex 50×2-200 ion exchange resin (strong acid; TsOH residues) in 2,2-dimethoxypropane at 83 °C. After hot filtration of the resin, **10** could be crystallised as described for the 1 kg work. Another advantage was that all crops were less coloured than when using TsOH since the coloration occurred only on the resin. This, however, did not prevent its reuse.[12] Typical yields were 80−90% including a second crop. This methodology was used routinely with 57 kg input of diol **9**. Requirements for 100 kg quantitites of final product **11** were satisfied using **10** that had been prepared using Dowex 50×2-200 ion exchange resin. In the next phase of work the ethylamine amidation of **10** was scaled to a 20 L pressure vessel with typically 4.75 kg of **10** in 9.5 kg of anhydrous ethylamine. Five reactions were performed in the presence of 3.6 mol % ethylamine hydrochloride. The effects of this acid catalyst were quite dramatic, with the reaction being complete in 3 h.

The significant demonstration of acid catalysis and the need to scale the conversion of **10** to **7** to standard pilot plant vessels prompted investigation into conducting the chemistry at atmospheric pressure. It also appeared attractive to use aqueous ethylamine in order to reduce the costs of handling the anhydrous material on pilot plant scale. Indeed it was discovered that the amidation of **10** can be carried out using 70% aqueous ethylamine in propan-2-ol in the presence of ethylammonium chloride (10 mol %) under conditions of reflux for 15 h. In this particular case ethylammonium ion was found to be essential for significant reaction to occur over this time period. Concentration under vacuum left a residue of amine **7**. Unfortunately, up to 10% lactam hydrolysis occurred over the period of reaction, which was avoided by utilisation of anhydrous ethylamine in the appropriate cosolvent. These procedures offered significant advantages over our earlier process since high pressures and temperatures are circumvented, thus avoiding the need for costly pressure apparatus.

Process Improvements to the Amidation of Lactam Acetonide 10 and Isolation of Final Product 11. A further extension of the acid-catalysed amidation of **10** was to use 1 molar equiv of ethylammonium benzoate and 2 molar equiv of anhydrous ethylamine at reflux in THF. The reaction progressed cleanly and conveniently to the benzoic acid salt **11**, and at the point of complete reaction, all of the ethylammonium benzoate was consumed. Further exploratory chemistry has led to the process whereby free ethylamine is present in catalytic amounts only (5 mol %) and ethylammonium benzoate provides the stoichiometric source of ethylamine. Although significantly slower, 80% reaction was achieved in THF under conditions of reflux over 24 h. Upon cooling the solution, the salt **11** crystallised with ease.

Residual Osmium Analysis of Compounds 10 and 11. The benzoic acid salt **11** is an advanced intermediate for the synthesis of potential pharmaceutical products. The residual osmium levels in acetonide **10** and final product **11** were important considerations for obvious reasons of toxicity. Residual osmium levels in the acetonide **10** were 12−20 ppm and 110−200 ppm for first and second crops,

(11) A second catalytic cycle is suggested leading to a stable osmium diglycolate which forms slowly over time. See the following references: (a) Kolb, C. H.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483. (b) Wai, J. S. M.; Marko, I.; Svenden, J. S.; Finn, M. G.; Jacobsen, E. N.; Sharpless, K. B. *J. Am. Chem. Soc*. **1989**, *111*, 1123.

(12) The resin could be treated as described in the Experimental Section for lactam acetonide **10** (wash cycle), which would return the resin to a pale yellow colour. The resin could be used repeatedly.

respectively. Typically the osmium levels in the crude salt **11** were <10 ppm, which after recrystallisation reduced to <1 ppm.

Conclusions

The production of the carbocyclic nucleoside precursor **7** at pilot scale has presented us with several synthetic challenges. Notably the key osmium tetraoxide dihydroxylation of **8** has been developed to the extent that the procedure reported herein could be applicable to dihydroxylation processes with other olefinic substrates.

The amidation reaction to prepare **7** previously reported[5] has been shown to be highly dependent upon acid catalysis to the extent that the otherwise high-energy noncatalysed lactam ring opening of **10** with alkylamine can be reduced to a simple atmospheric pressure reaction where stoichiometric alkylamine nucleophile is derived *in situ* from 1 molar equiv of its conjugate acid alkylammonium ion. The stoichiometric benzoate in our particular case served as a counterion for the key intermediate **7**, thus facilitating manufacture of a stable and highly crystalline salt **11**.

Experimental Section

(1*R*,4*S*,5*R*,6*S*)-2-Azabicyclo[2.2.1]-5,6-dihyroxyheptan-3-one (9). *General Developed 1 kg Scale Procedure*. To a solution of **8** (1 kg, 9.17 mol) in acetone (5.7 L) heated to gentle reflux was added osmium tetraoxide (2.33 g, 9.17 mmol) as a 4% solution (58 mL). A 50% aqueous solution of NMO (2.14 L equating to 1.2 kg of NMO, 10.3 mol) was added under conditions of reflux over 2.5 h. After a further 0.5 h the solution was cooled to 20 °C. Sodium bisulphite (50 g) in water (150 mL) was added and the mixture filtered through a bed of talcum powder (0.38 kg). The filtrate was concentrated as far as possible to a viscous oil. Methanol (3 L) was added and the mixture heated until dissolution. The liquor was filtered and then allowed to crystallise at 5− 10 °C. After 2−3 h the crop was filtered off, washed with methanol (0.5 L), and dried to a grey/white crystalline solid, 1.1 kg (83%).

Pilot Plant Work. The above method was employed using **8** (21.5 kg, 197 mol). The work was carried out in a 200 L glass-lined Balfour reactor fitted with a 50 L measure head tank and coupled with a 100 L glass receiver vessel. Talcum powder was added to the vessel and removed by filtration through an in-line filter bag assembly. All solvent removal was carried out in the Balfour using recirculating water at 60−70 °C. Typical yields were 23 kg, 79% single crop: purity >95% by HPLC;[13] ^{1}H NMR 270 MHz ($CDCl_3$/DMSO-d_6) δ 0.79 (d, 1 H, CH_2), 1.83 (d, 1H, CH_2), 2.39 (s, 1H, CHCO), 3.52 (s, 1H, CHN), 3.82 (bm, 1H, CHOH), 3.89 (bm, 1H, CHOH), 4.95 (d, 2H, OH), 7.42 (s, 1H, NH).

[3a*S*,4*R*,7*S*,7a*R*]-Tetrahydro-2,2-dimethyl-4,7-methano-1,3-dioxolo[4,5-*c*]pyridin-6(3a*H*)-one (10). The following procedure was used routinely on pilot plant scale with a 250 L glass-lined vessel. Dowex 50×2-200 ion exchange resin (8 kg) was prepared for use by washing several times in accordance with the manufacturer's notes (1 M NaOH × 2, water × 2, 1 M HCl × 2, water × 2; all 16 L aliquots). The resin was finally washed with acetone (5 L × 2). The diol **9** (57 kg, 400 mol) was charged to the 250 L vessel. The prepared resin (5.5 kg) was charged followed by 2,2-dimethoxypropane (186 L). The suspension was heated to reflux until a clarified solution was evident and the resin blackened. The mixture was filtered whilst hot through an in-line stainless steel bag filter assembly. After transfer of the filtrate back to the cleaned vessel, the solution was allowed to cool to <10 °C and the first crop collected and washed with acetone (40 kg). After drying, the yield was 44 kg, 61%. All liquors and washings were concentrated to <20% of the original volume. A further quantity of acetone was added (20 kg). The second crop was harvested after 2−3 h and washed with acetone (10 kg). The yield after drying was 14 kg, 20%: mp 140−141 °C; ee >99%; purity >98% by HPLC;[13] ^{1}H NMR (see structure **10** for atom assignment) 270 MHz ($CDCl_3$) δ 1.36 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 2.06 (dt, 1H, CH_2), 2.12 (dt, 1H, CH_2), 2.72 (s, 1H, H7), 3.79 (s, 1H, H4), 4.41 (d, 1H, H3a), 4.54 (s, 1H, H7a), 6.67 (bs, 1H, NH). Anal. Calcd for $C_9H_{13}NO_3$: C, 59.00; H, 7.15; N, 7.64. Found: C, 59.07; H, 7.44; N, 7.64.

[3a*R*,4*S*,6*R*,6a*S*]-6-Amino-*N*-ethyltetrahydro-2,2-dimethyl-4*H*-cyclopenta-1,3-dioxole-4-carboxamide (7). *Pressure Reaction.*[5] The protected diol **10** (4.75 kg, 25.9 mol) was dissolved in predistilled anhydrous ethylamine (9.5 kg, 210 mol) containing ethylamine hydrochloride (75 g, 0.92 mol) in a 20 L pressure vessel. The solution was heated to 120 °C under pressure (220 psi, 10 bar) for 3.5 h. Following cooling to 20 °C the ethylamine solution was transferred to a 65 L glass-lined vessel and excess solvent removed under vacuum.

Anhydrous Ethylamine at 1 atm. The protected diol **10** (91.5 g, 0.5 mol) was suspended in THF (500 mL). Ethylamine (100 mL, 1.53 mol) was added with stirring at 5 °C. Benzoic acid (61 g, 0.5 mol) in THF (100 mL) was added at this temperature over 30 min. The solution was brought to reflux for 18 h (internal temperature 55−60 °C). The solution was concentrated under vacuum until precipitation occurred. After cooling to 10 °C, the salt was filtered off and washed with THF (50 mL). The liquors were concentrated, which allowed crystallisation of a second crop. The total yield was 140 g, 80%. All crops were recrystallised from propan-2-ol (300 mL). The yield was 115 g, 65%, first crop, and 9 g, 5%, second crop; total 124 g, 70%: ^{1}H NMR (see structure **7** for atom assignment) 270 MHz ($CDCl_3$/DMSO-d_6) δ 1.13 (t, 3H, Et CH_3, J = 7.29 Hz), 1.29 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.80 (dt, 1H, CH_2, J = 13.0, 4.2 Hz), 2.00 (d, 1H, CH_2, J = 13.0 Hz), 2.40 (m, 1H, H4), 3.26 (m, 2H, Et CH_2), 2.78 (m 1H, H6), 3.45 (bm, 1H, NH), 4.35 (d, 1H, H3a, J = 5.5 Hz), 4.83 (dd, 1H, H6a, J = 5.5, 3.0 Hz).

Benzoic Acid Salt of 7: Product 11. To the residual viscous crude amide **7** (5.9 kg, 25.9 mol) was added propan-2-ol (13 L). Benzoic acid (3.13 kg, 25.9 mol) was dissolved in propan-2-ol (12 L). This solution was added over 2−3 h to the stirring solution of **7** at 25 °C. When crystallisation was evident, the suspension was heated to allow dissolution at reflux. The hot solution was filtered through an in-line 10 μm cartridge filter into a crystallisation vessel. After cooling to 10 °C, the crop was filtered off, washed with

(13) Kromasil reverse phase 5C8 150 × 4.6 mm column with 50% MeOH in 10 mM K_2HPO_4 at pH 7.0, flow 1.0 mL/min and detection at 225 nM.

propan-2-ol (5 L), and dried at 40 °C under vacuum to yield 8.0 kg of **7**, 90%. Several crops of **8**, 45 kg were recrystallised from propan-2-ol, 78 L. The above procedure was used routinely on pilot plant scale using a 200 L glass-lined vessel: mp 167 °C dec; de >99%, ee >99%[9]; ^{1}H NMR (see structure **7** for atom assignment) 270 MHz ($CDCl_3$/DMSO-d_6) δ 1.09 (t, 3H, Et CH_3, J = 7.56 Hz), 1.22 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 2.00 (d, 1H, H5, J = 14.8 Hz), 2.40 (dt, 1H, H5, J = 14.8, 8.1 Hz), 3.01 (d, 1H, H4, J = 8.3 Hz), 3.18 (m, 2H, Et CH_2), 3.70 (d, 1H, H6, J = 6.8 Hz), 4.71 (s, 2H, H3a, H6a), 7.40 (m, 3H, Ph), 8.01 (d, 2H, Ph), 8.30 (bs, 1H, NH). Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.83; H, 7.67; N, 7.96.

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