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Synthesis and in vitro stability of amino acid prodrugs of 6-β-naltrexol for microneedle-enhanced transdermal delivery

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ABSTRACT

A small library of amino acid ester prodrugs of 6-β-naltrexol (NTXOL, **1**) was prepared in order to investigate the candidacy of these prodrugs for microneedle-enhanced transdermal delivery. Six amino acid ester prodrugs were synthesized (**6a**–**f**). **6b**, **6d**, and **6e** were stable enough at skin pH (pH 5.0) to move forward to studies in 50% human plasma. The lead compound (**6e**) exhibited the most rapid bioconversion to NTXOL in human plasma ($t_{1/2}$ = 2.2 ± 0.1 h).

A recent surge in research interest has focused on the design of amino acid prodrugs as delivery systems. The strategy has been applied to oral,[1] intraocular,[2] intranasal[3] and intravenous[4] therapeutic agents which exhibit suboptimal physiochemical properties that limit drugability. Generally, the goal of amino acid prodrug design is to target nutrient transporters at various biological barriers; however, at skin-relevant pH 5.0, the free amine of an amino acid promoiety can also enhance aqueous solubility. Indeed, amino acid promoieties have already been explored as solubility-enhancing agents in the design of injectable metronidazole prodrugs.[5,6] In passive transdermal delivery systems, where intact stratum corneum (SC) limits the permeability of hydrophilic molecules, ionizable amine promoieties would be expected to diminish molecular permeability of a prodrug. However, the use of microneedles to create skin microchannels followed by the application of a drug-containing transdermal formulation allows for delivery of hydrophilic species.[7] This method is referred to in this article as microneedle-enhanced transdermal delivery (MN) and is more specifically known as the 'poke and patch' technique. Therefore, our goal in this study was to synthesize amino acid prodrugs of NTXOL (**2**) and to investigate their stability properties in buffers and in human plasma (HP), in order to predict MN candidacy. The field of MN is relatively new,[8] and to our knowledge, there are currently few examples of amino acid prodrugs in the literature which are intended for MN.[9]

MN is a technique in which small microchannels are created in the skin with micrometer-sized needles. SC is bypassed in this way, and as a consequence of this, MN has expanded the pool of molecules that can be delivered by the percutaneous route. Improved skin transport of hydrophilic or charged compounds,[10–12] and molecules with large molecular weights in the kilodalton range[13] has been observed utilizing MN. For instance, transdermal delivery of large proteins[14] and hydrophilic compounds such as calcein[8] have been enhanced by MN. Previously, work in our labs demonstrated that the FDA-approved opioid antagonist naltrexone (NTX, **1**) and its active metabolite, 6-β-naltrexol (NTXOL, **2**) could not be delivered in therapeutic levels by passive transdermal delivery techniques.[15] Accordingly, attempts were first made to manipulate the physiochemical properties of NTX by the prodrug approach. Straight-chain and branched-chain alkyl esters and carbonates of NTX were prepared, but these prodrugs achieved suboptimal skin diffusion for therapeutic delivery in man.[16–20] Therefore, it was envisaged that a switch to MN, and the use of more hydrophilic amino acid prodrugs of **2**, might be an appropriate strategy. NTXOL is attractive for this research, because it is thought to be instrumental in the therapeutic effects of orally dosed NTX. Also, **2** has an aliphatic hydroxyl group, which is not present in the NTX molecule that is more suitable for the design of ester prodrugs. We postulated that identification of a lead NTXOL prodrug for further MN studies could eventually be advantageous to improve the

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therapeutic outcomes of patients during protracted alcohol and/or opioid cessation efforts, since current forms of NTX therapy are associated with adverse events that lead to noncompliance.[21] In this respect, we have recently demonstrated that pegylated prodrugs of NTX, while more soluble in 0.3 M acetate buffer than NTX, unfortunately exhibit problematic viscosity properties that limit microchannel transport when compared to NTX itself.[22]

Amino acid ester prodrug design has been approached in this study as an alternative strategy to improve molecular hydrophilicity while simultaneously avoiding the creation of a viscous and oily prodrug material. Herein we describe the synthesis and stability of amino acid ester prodrugs of **2**. Among the compounds mentioned, **6e** is the established lead compound for further MN developmental work.

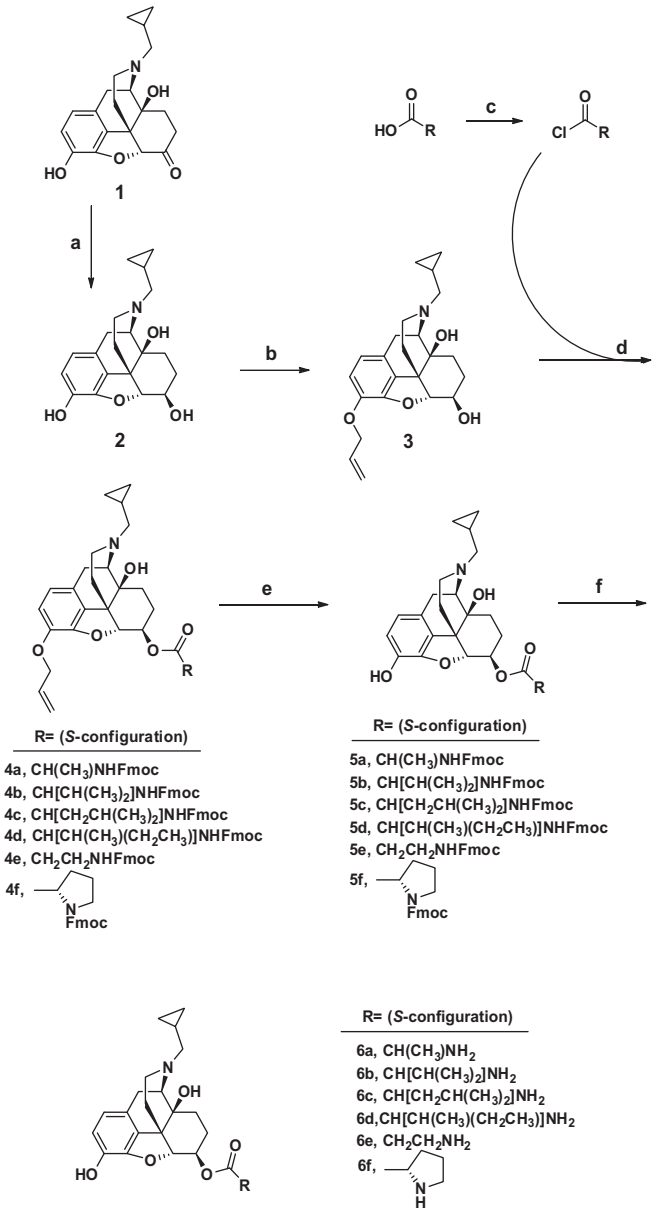
It is known that amino acid ester prodrugs can vary widely in their stability properties in various hydrolytic media (enzymatic and non-enzymatic), depending on molecular factors and on the pH of the hydrolysis media. Skin pH is around 5.0 on average. Thus, we set the criterion that no produg could degrade more than ten percent within the intended forty-eight hour time course of future skin diffusion studies that would be conducted in 0.3 M acetate buffer vehicle (i.e. $t_{90} \geqslant 48$ h at pH 5.0). Also, since a prodrug must hydrolyze in vivo to release parent drug, rapid hydrolysis at pH 7.4, with or without enzyme catalysis, was established as an important physiochemical parameter. Prodrugs exhibiting a combination of these features were considered to be appropriate molecules for further drug development.

Amino acid esters of **2** were prepared as depicted in Scheme 1. NTXOL (**2**) was afforded in 85–92.7% yield (depending on scale) from NTX using the synthetic methodology of de Costa et al.[23] 3-*O*-allyl-NTXOL (**3**) was prepared by treating **2** with allyl bromide under reflux in acetone in the presence of potassium carbonate. Excess allyl bromide was washed out with hexanes. For each coupling reaction, **3** and DMAP were dissolved at room temperature in DCM. The appropriate Fmoc-protected amino acid promoiety was converted to an activated acid chloride in a separate flask by sonicating it in DCM with thionyl chloride under an argon stream, as described by Sureshbabu et al.,[24] and the residual HCl that had not been blown off in the argon stream was quenched with DMAP base. The cocktail containing **3** and DMAP was then added dropwise to the acid chloride solution over the course of two minutes at 0 °C, and the reaction mixture was warmed to room temperature and left to react for four hours.

In every case, the reactions were worked up with DI water and brine. Final purification was achieved by chromatography over silica that had been pretreated with 1% TEA in hexanes. Isolated precursors (**4a**–**f**) were used without further purification. Estimated isolation yields were 75–90%.

Deallylation was performed utilizing the methods of Chandrasekhar et al.[25] with modifications. The fully protected precursor was dissolved in THF and treated with *tetrakis*(triphenylphosphine)palladium ($(PPh_3)_4Pd$) and poly-methylhydrosiloxane (PMHS). Zinc chloride (2 M solution in diethyl ether) was added with rapid stirring, and the reaction was run for 24 h. The THF was evaporated under an argon stream, and the gummy residue was reconstituted in DCM and worked up with a 1% sodium bicarbonate solution and brine. The combined organic fractions were concentrated and chromatographed as before to afford the deallylated prodrug product in estimated yields ranging from 50% to 85%. Compounds **5a**–**f** were advanced to the next step without further purification.

Fmoc removal was accomplished by 24 h treatment with DBU base (25 mol %) and octanethiol (10 equiv) in dry THF, as described by Sheppeck et al.[26] In the case of **6e**, the final prodrug was isolated by precipitation and trituration from diethyl ether. The α-amino acid ester prodrugs were recovered via aqueous workup and column chromatography, utilizing silica deactivated with 1% TEA in hexanes,



Scheme 1. Synthesis of amino acid ester prodrugs of 6-β-naltrexol: (a) formamidinesulfinic acid (4.0 equiv), aqueous NaOH (0.53 M), 80 °C, 1.5 h;[23] (b) allyl bromide (1.1 equiv), K_2CO_3 (4 equiv), acetone, reflux, 5.5 h; (c) Fmoc-protected amino acids (2.0 equiv), $SOCl_2$ (1.99 equiv), sonicate 30 min, DMAP (2.0 equiv), argon stream; (d) DMAP (2.0 equiv), DCM, 0 °C to rt, 4 h, argon atmosphere; (e) PMHS (3.0 equiv), 2 M $ZnCl_2$ in Et_2O (52 drops via syringe/100 mg **2**), $(PPh_3)_4Pd$ (5 mol %), THF, rt, 24 h, argon atmosphere; (f) octanethiol (10 equiv), DBU (25 mol %), THF, rt, 24 h, argon atmosphere.

and the final prodrugs were eluted with copious column washes using ethyl acetate. Compounds **6a** and **6f** required acetone and TEA to be eluted from silica, and hydrolytic degradation of these prodrugs resulted. The range of yields at this stage was ~20% (**6a**) to 80% (**6d**). The final prodrugs were characterized by ^{1}H NMR and ^{13}C NMR spectrometry, and ESI-MS analysis.[31] Final yields of prodrugs are reported along with the spectral data. Despite some degradation during purification, compounds **6a** and **6f** were obtained in sufficient purity for stability comparison to the other prodrugs.

For prodrugs **6a**–**f**, stability studies were conducted in donor vehicle (0.3 M acetate buffer, pH 5.0). For prodrugs **6b** and **6e**, stability studies were also conducted in receiver solution (25 mM HEPES-buffered Hanks' balanced salt solution, pH 7.4). These donor