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# Synthesis and immunological effects of heroin vaccines containing haptens with improved stability

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**Absract:** Three haptens have been synthesized with linkers for attachment to carrier macromolecules at either the piperidino-nitrogen or via an introduced 3-amino group. Two of the haptens, with a 2-oxopropyl functionality at either C6, or at both the C3 and C6 positions on the 4,5-epoxymorphinan framework, as well as the third hapten (DiAmHap) with diamido moieties at both the C3 and C6 positions, should be much more stable in solution, or *in vivo* in a vaccine, than a hapten with an ester in one of those positions, as found in many heroin-based haptens. A "classical" opioid synthetic scheme enabled the formation of a 3-amino-4,5-epoxymorphinan which could not be obtained using palladium chemistry. Our vaccines are aimed at the reduction of the abuse of heroin and, as well, at the reduction of the effects of its predominant metabolites, 6-acteylmorphine and morphine. One of the haptens, DiAmHap, has given interesting results in a heroin vaccine and is clearly more suited for the purpose than the other two haptens.

**Key words:** hapten, isostere, 3-amino-4,5-epoxymorphinans, 3,6-diacetamido-4,5-epoxymorphinans, vaccine, heroin, DiAmHap

## Introduction

In the last several years, vaccines have been increasingly targeted as a possible treatment modality for heroin abuse,[1-12] or the abuse of other opiates.[4] Many of those that been mentioned in the literature have used heroin-like haptens or haptens with a labile ester functionality at either C3 or C6 in a 4,5-epoxymorphinan structure. In our search for haptens that would offer greater stability in solution than compounds with heroin-like ester functions at C3 or C6, offering increased shelf life when formulated in a vaccine and stability *in vivo*, we sought different types of substituents at C3 and C6 in the 4,5-epoxymorphinan template. We believed, using the Matyas et al., concept of facial recognition,[12] that



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haptens (Fig.1) based on these compounds could be used in a vaccine that would preferentially interact with heroin and some of its metabolic products.

Two different isosteric approaches were conceptualized for modification of the template's substituents. In one approach we envisioned replacement of the ester function in heroin with a similar-appearing relative, a 2-oxopropyl moiety at C6 (*N*-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-methyl-7-(2-oxopropyl)-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-9-yl)-3-(tritylthio)propanamide, **1**, 6-PrOxyHap, Fig. 1), as well as 2-oxopropyl moieties at both C3 and C6 in a second hapten (*N*-(4-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-7,9-bis(2-oxopropyl)-4,4a,5,6,7,7a-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-3(2*H*)-yl)butyl)-3-(tritylthio)propanamide, **2**, DiPrOxyHap, Fig. 1). In these compounds the oxygen atom in the acetyl group of heroin was replaced with a methylene group. In a second approach we envisioned the use of an amido function at both C3 and C6 that is present at C3 in hapten **1**. This became our third hapten target, *N*,*N*'-((4*S*,4a*R*,7*S*,7a*R*,12b*R*)-3-(4-(3-(tritylthio)propanamido)butyl)-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline-7,9-diyl)diacetamide (DiAmHap, **3**, Fig. 1**)**. Both the 2-oxopropyl and the amido moieties in a 4,5-epoxymorphinan structure have reasonably similar facial similarity to heroin, and both are inherently more stable than ester functionality at those C3 and C6 positions.

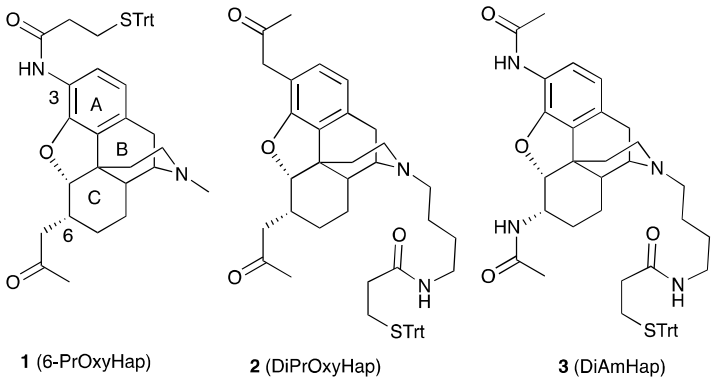


**Fig. 1** Target haptens

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These three haptens were coupled to tetanus toxoid mixed with liposomes for immunization and high titer antibodies were induced. The ability of the anti-hapten antibodies to bind to heroin, 6-acetylmorphine and morphine was determined by competitive ELISA experiments, and mice were immunized to examine the antinociceptive effects of injected heroin by hot plate assays.

## Results and discussion

### Synthesis of haptens 1-3

There were two key elements for the synthesis of the three haptens (**1-3**, Fig. 1). One was installation of a 3-amino group and another was the stereoselective construction of a C-6α-(2-oxo-propyl) group. Because of the preexisting C3-hydroxyl group in the starting material, we initially considered direct conversion of a phenol to an aniline. Among the possible routes, high temperature Smiles rearrangement was not suitable because of the instability of the opiate structure. Palladium-catalyzed amination would be an efficient route for conversion of phenols to anilines after activation of the phenolic hydroxyl group by conversion to its corresponding sulfonate.[13] However, the success of this methodology seems to be substrate-dependent in 4,5-epoxymorphinan scaffolds.[14-16] The palladium-catalyzed C-N coupling reaction worked sluggishly on the protected morphine derivatives under varied conditions[5, 14, 15] and did not work at all on the oxymorphone skeleton, even when the C-6-ketone was protected as the ethylene glycol ketal.[16] It is unclear whether the carbonyl group or the conformation of the C-ring interfered with the formation the metal-ligand-substrate complex.

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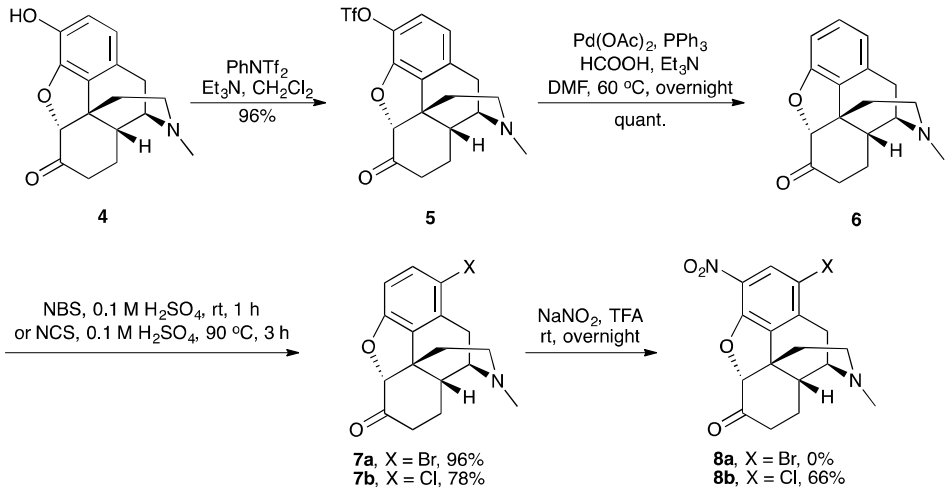


**Scheme 1** Introduction of the 3-nitro substituent

A circuitous route containing a 5-step synthetic sequence was developed (Scheme **1**) starting from hydromorphone. Conversion of the 3-phenolic hydroxyl group to the triflate, followed by palladium-catalyzed reduction[15] furnished 3-desoxy-hydromorphone **6**[17] in 96% yield over two steps. Regioselective nitration at the C3 position necessitated selective blocking of the more reactive C1 position. We initially selected bromine as a labile protecting group. The selective bromination at C1 proceeded smoothly using NBS at room temperature in 96% yield and none of the C3 brominated product was observed.[18] Nitration of **7a** under mild conditions ($NO_2BF_4$) did not succeed and only starting material **7a** was recovered, while more forcing conditions ($HNO_3$, $H_2SO_4$ or $NaNO_2$, TFA) gave rise to a mixture of by-products. We noted[19] that in a system related to **7a**, a brominated intermediate was not suitable because of *ipso* nitration and the resulting migration or loss of bromide. We decided to replace the bromide in **7a** with a more stable halide. The chloride **7b** was prepared under similar conditions as **7a**, using NCS as the chlorine source in 78% yield, and it proved to be stable under the nitration conditions. Optimal nitration occurred with $NaNO_2$-TFA at 0 $^oC$, providing the desired product **8b** in good yield. The regio-selective chlorination on C1 and nitration on C3 was unambiguously confirmed by single crystal X-ray crystallographic analysis of compound **8b** (Fig. **2**).

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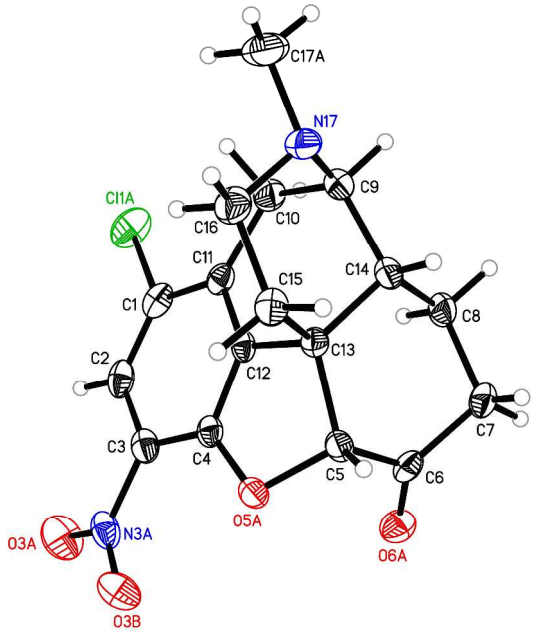
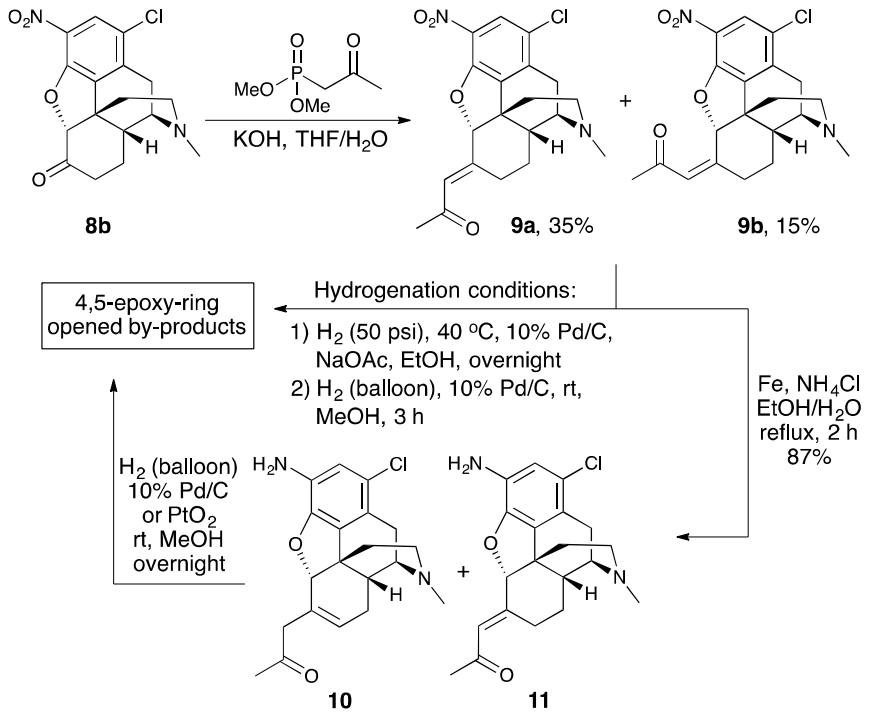



**Fig. 2** Structure of **8b** from X-ray crystallographic analysis

With the nitro product **8b** in hand, we turned to the introduction of the C-6-substituent (Scheme **2**).




**Scheme 2** Attempted introduction of the C6α 2-oxopropyl group at C6

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Compound **8b** was treated with dimethyl 2-oxopropyl-phosphonate to give a mixture of *E*- (**9a**) and *Z*-isomers (**9b**) in 36% and 15% yields, respectively, after repeated chromatographic purification. The less polar compound **9a** was assigned as the *E*-isomer by $^1$H NMR because of the existence of a vinyl proton with a chemical shift of 6.55 ppm. A vinyl proton in a similar compound was noted to have a chemical shift of 6.50 ppm.[20] The vinyl proton was found at 5.86 ppm in the $^1$H NMR of **9b**. The vinyl proton in the *E*-isomer **9a** was seen at lower field presumably because of the deshielding effect of the oxygen atom in the 4,5-epoxy ring.

A palladium-catalyzed hydrogenation (Scheme 2) was attempted to stereoselectively reduce the α, β-unsaturated ketone with concomitant cleavage of the C1 chlorine and reduction of the C3 nitro group in the **9a** + **9b** mixture. Various hydrogenation conditions were attempted, using high or ambient pressure, room temperature or higher temperatures, and with different solvents and catalysts. Unfortunately, the desired aromatic amine could not be obtained under any of these conditions. Only the 4,5-epoxy-ring opened by-products with or without chlorine or the C6-C7 double bond was isolated from the reactions.

We presumed that the strongly electron-withdrawing nitro group could be a factor leading to the failure of the reduction. Thus, the nitro group was reduced smoothly to an electron-donating amino group with Fe/$NH_4Cl$, in 88% yield. However, due to partial isomerization[20] of the α, β-unsaturated ketone to the β, γ- unsaturated ketone, a mixture of **10** and **11** was obtained and only the less polar isomerized product **10** could be isolated as a pure product. The transposition of the double bond was confirmed by the existence of a vinyl proton doublet with a chemical shift of 5.69 ppm in the $^1$H NMR spectrum of **10**. Catalytic hydrogenation of the mixture of compounds **10** and **11** gave 4,5-epoxy ring-opened by-products. All other attempts (dissolving metal,[21] $[(Ph_3P)CuH]_6$[22] with or without $Et_3SiH$, DIBAL + CuMe,[23] and transfer hydrogenation[24]) to reduce the double bond were unsuccessful.

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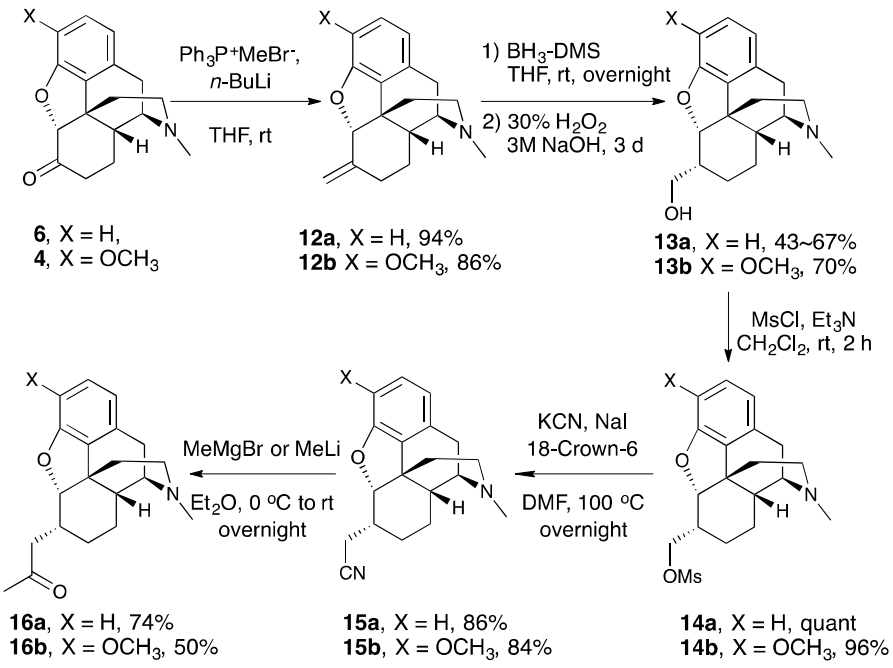
Chadha and Rapoport have reported a successful Pt-catalyzed reduction of a C-6 methylenic 4,5-epoxymorphinan compound[25] to afford a C6α-methyl side chain. Also, dimide was reported[26] to reduce a C6 exocyclic double bond with a larger C6 side chain, giving a β-orientated configuration. Theoretically, metal (Pd and Pt) catalytic hydrogenation should be facile due to formation of a π-allylic cation metal complex of the allylic ether. Unfortunately, with our substrates, the use of metal catalysts gave us phenolic by-products from concomitant opening of the 4,5-epoxy ring. Thus, metal catalyzed reduction could not be used to construct the C-6α side chain, and a new route to the 2-oxopropyl side-chain at the C-6α position was sought.

This route (Scheme 3) was initiated from 3-desoxy-hydromorphone **6**, which was converted to 6-methylene-4,5-epoxymorphinan **12a** in 94% yield via a Wittig reaction.[25] A hydroboration-oxidation reaction sequence[27] was employed to stereoselectively convert the 6-methylene to a 6α-hydroxymethyl compound **13a**, via attack of borane from the less hindered *exo* face in moderate to good yield.




**Scheme 3** Construction of the C6α 2-oxopropyl side-chain



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The configuration of the C6 side chain in **13a** was suggested by NMR (the coupling constant $J_{5,6}$ in a compound with a C-6α-orientated side chain is smaller than that in a compound with a C-6β-orientated side chain[28]). It was verified by X-ray crystallographic analysis (**Fig 3**).

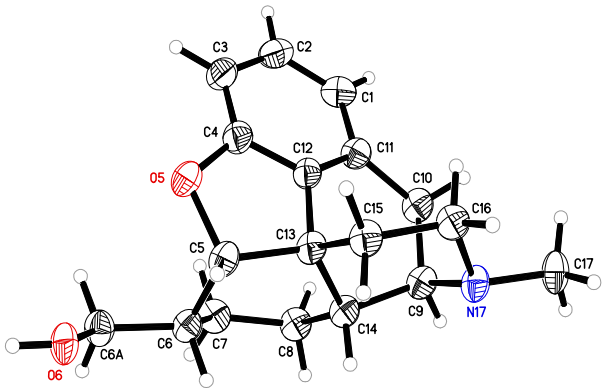


**Fig 3** Structure of **13a** from X-ray crystallographic analysis

Mesylation of the hydroxyl group in **13a** gave **14a** in quantitative yield (Scheme 3), which was converted to nitrile **15a** in 86% yield by displaced with potassium cyanide. Nitrile **15a** was treated with MeLi or MeMgBr in $Et_2O$. $Et_2O$ is crucial to the success of this reaction. Either THF or a mixture of THF and $Et_2O$ failed to give the desired ketone. This was followed by hydrolysis of the intermediate imine with HCl to give the ketone **16a** in 74% yield. Compound **16b** incorporating a C3-methoxy was synthesized using the same sequence from hydrocodone **4**.

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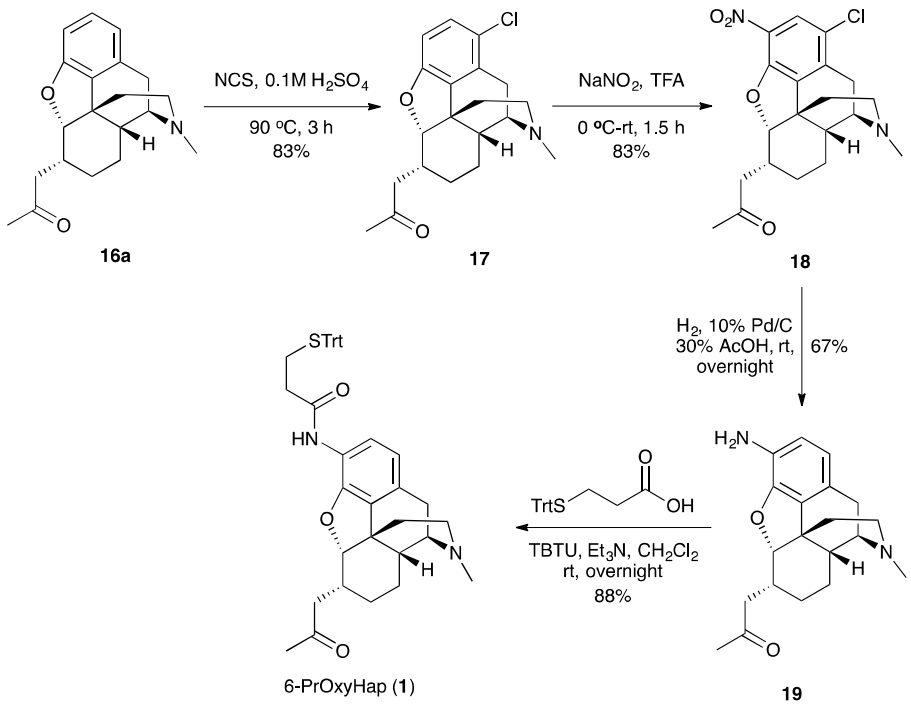


**Scheme 4** Completion of the synthesis of 6-PrOxyHap

The synthesis of the target hapten **1** was completed starting from **16a** (Scheme **4**). Nitro product **18** was obtained in 83% yield over 2 steps using the same chlorination/nitration protocol as for compound **8b**. Simultaneous removal of the chlorine atom and reduction of the nitro group proceeded smoothly under palladium-catalyzed hydrogenation in aqueous acetic acid and afforded aniline **19** in 67% yield. Coupling of the aniline **19** and 3-(tritylthio)propanoic acid using TBTU yielded the target 6-PrOxyHap **1** in 88% yield. From the intermediate **16b** (Scheme **5**), 3-*O*-demethylation with $BBr_3$ yielded phenol **20** in 85% yield[29] and reaction of phenol **20** with $Tf_2NPh$ gave triflate **21** in 91% yield.

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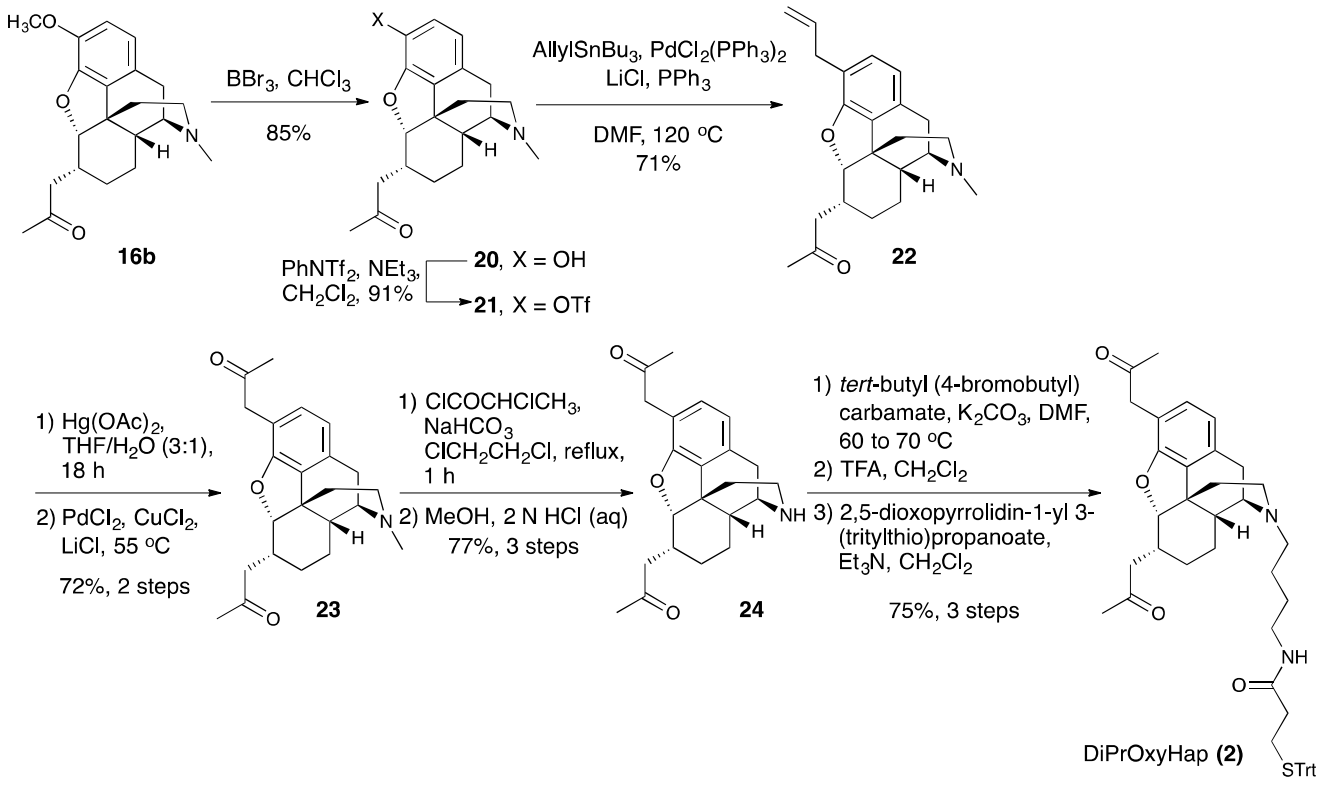


**Scheme 5** Synthesis of DiPrOxyHap (**2**)

A Stille coupling reaction[30] of **21** and allyltributyltin gave alkene **22** in 71% yield. A modified two-step Wacker-type oxidation[31] with a selective oxymercuration, transmetalation and oxidation sequence gave the methyl ketone **23** in 72% yield. *N*-Demethylation with 1-chloroethyl chloroformate and aqueous hydrochloric acid gave the secondary amine **24** in 77% yield. Alkylation with *tert*-butyl (4-bromobutyl) carbamate followed by deprotection and amidation with 2,5-dioxopyrrolidin-1-yl 3-(tritylthio)propanoate, gave the DiPrOxyHap (**2**) in 75% yield over three steps. The syntheses of 6-PrOxyHap **1** and DiPrOxyHap **2** were accomplished in 12 steps from hydromorphone and 14 steps from hydrocodone, respectively.

The synthesis of DiAmHap (**3**) was facilitated by the methodology employed in 6-PrOxyHap (**1**, Scheme 4), where the phenolic hydroxyl group was replaced by the C-3 amino functionality. We first turned our attention to the amino function at C-6. Reaction of 3-desoxyhydromorphone (**6**, Scheme 3)



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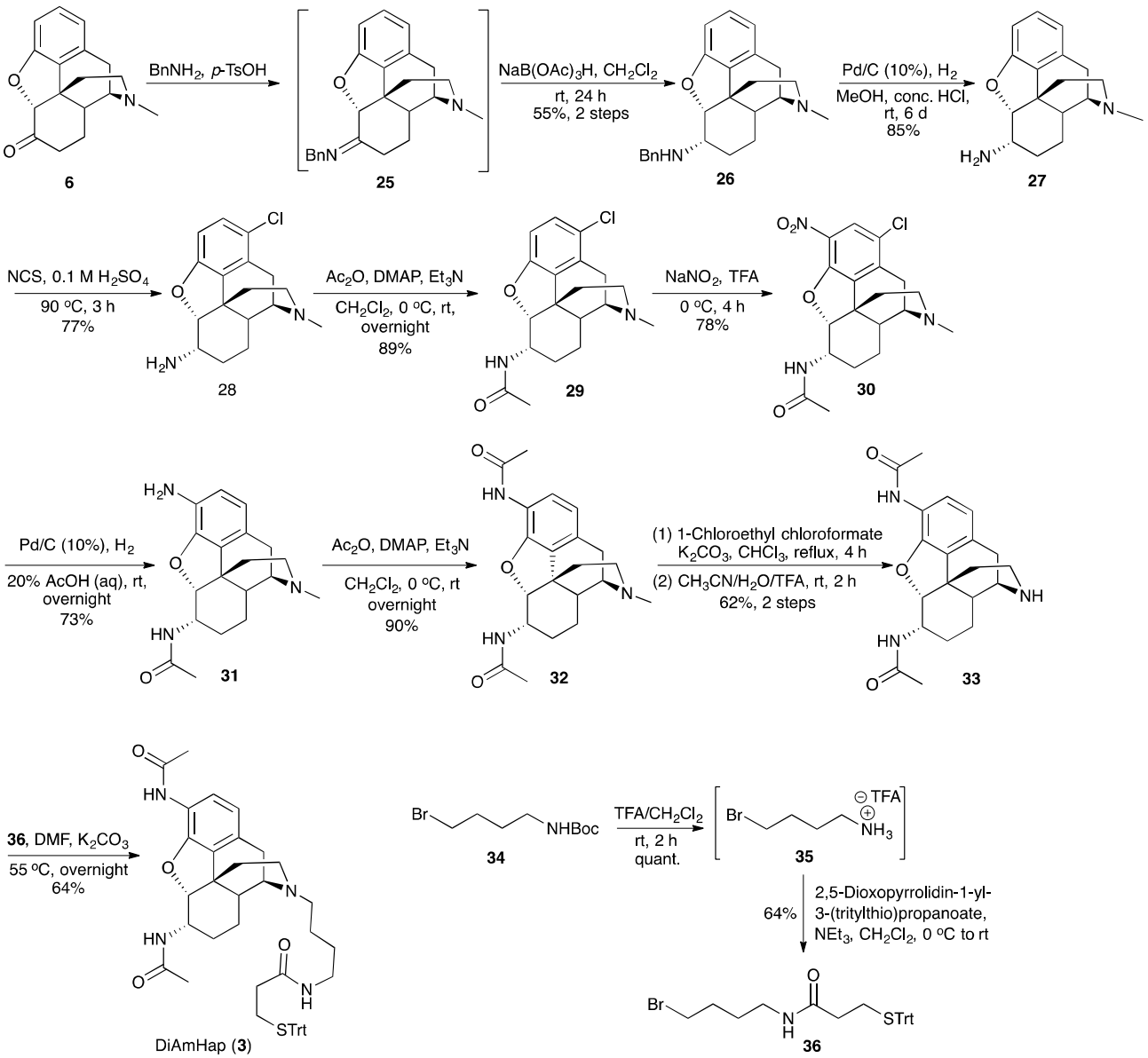
with benzylamine under dehydrating conditions followed by stereoselective reduction of the crude imine **25** provided the C-6α-benzylamine **26** in 55% yield for the two steps (Scheme 6).




Scheme 6 Synthesis of DiAmHap (**3**)

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Hydrogenolysis of the *N*-benzyl group in **26** over Pd gave the C-6α-amino derivative **27**, using methods analogous to those of Sayre and Portoghese[32]. The stereochemistry of **27** at C6 was proven through X-ray crystallography of the amine (Fig 4).

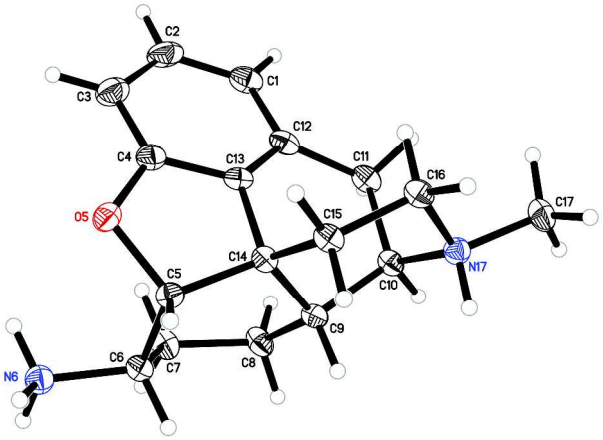


**Fig 4** X-ray crystal structure of (4*S*,4a*R*,7*S*,7a*R*,12b*R*)-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-amine (**27**)

Introduction of the amino moiety at C3 followed the procedure shown in Scheme 4. The C1-chloro compound **28** (Scheme 4) was prepared using N-chlorosuccinimide; the C6-amino moiety was then acetylated to give the C6-acetamide **29**. Nitration gave the C1-chloro, C3-nitro compound **30**. The nitro moiety at C3 was reduced to the C3-amine, removing the C1-chlorine atom in the process, to give **31**; acetylation gave the diacetamido compound **32**. The structure and stereochemistry of **32** was confirmed by X-ray crystallography (Fig. 5).

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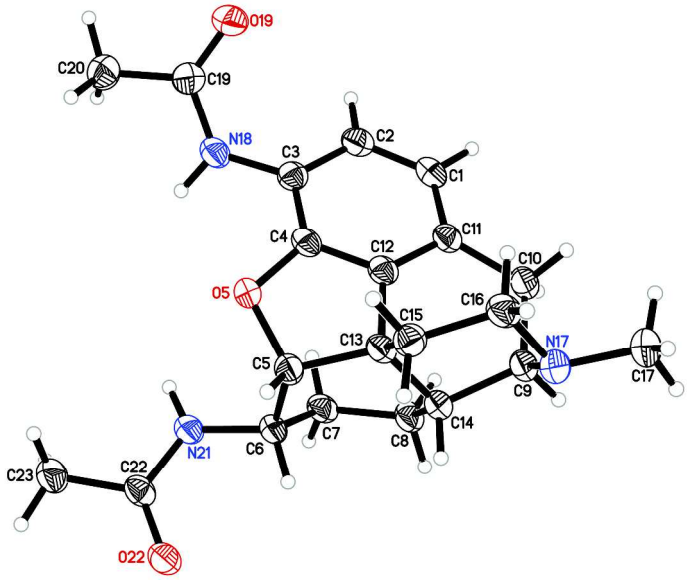


**Fig 5** X-ray crystal structure of *N*,*N*'-((4*S*,4a*R*,7*S*,7a*R*,12b*R*)-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline-7,9-diyl)diacetamide **32 (**DiAmHap precursor)

*N*-Demethylation of **32** to the secondary amine was accomplished using ACE chloride. Acetonitrile hydrolysis of the intermediate carbamate formed through reaction with 1-chloroethyl chloroformate, gave the *N*-nor derivative **33** in 62% overall yield. Reaction of **33** with the linker *N*-(4-bromobutyl)-3-(tritylthio)propanamide (**36**, Scheme 6, obtained by reaction of *tert*-butyl (4-bromobutyl)carbamate, **34**, with 2,2,2-trifluoroacetaldehyde, 4-bromobutan-1-aminium salt, **35**), gave DiAmHap **3**, in 64% yield. The synthesis of the biologically important compound DiAmHap **3** was achieved in 12 steps from hydromorphone (10 steps from 3-desoxyhydromorphone) with 10 characterized intermediates in about 6% overall yield.

## Biological Results

### Induction of antibodies

Immunization with the haptens coupled to tetanus toxoid mixed with liposomes containing MPLA induced high titer antibodies to each to the haptens. The average IgG endpoint titer to DiAmHap was



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3,000,000, while the titers to 6-PrOxyHap and DiPrOxyHap were 400,000 and 900,000, respectively (Fig. 5, week 9) (DiAmHap compared to 6-PrOxyHap and DiPrOxyHap, $p<0.0001$). Interestingly, the titers at 3 weeks after the primary immunization were approximately 10,000 for animals immunized each of the haptens (Fig. 5). These titers were maintained 6 weeks after the immunization for animal immunized with DiAmHap and 6-PrOxyHap, but dropped for animals immunized with DiPrOxyHap (DiAmHap compared DiPrOxyHap, $p = 0.001$-$0.01$ and 6-PrOxyHap compared to DiPrOxyHap, $p = 0.001$-$0.01$).

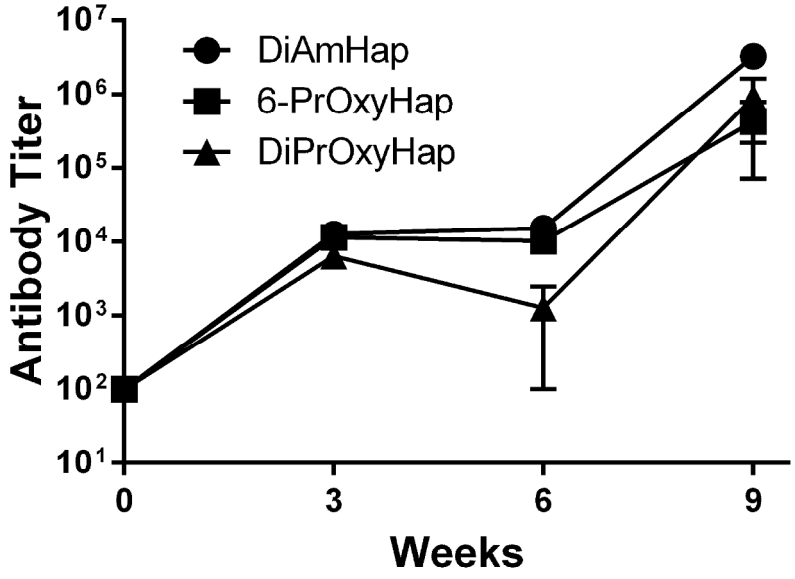


**Fig. 5** Time course of antibody response to DiAmHap, 6-PrOxyHap and DiPrOxyHap. Mice were immunized with the indicated hapten attached to TT at weeks 0 and 6. Animals were bled at the time indicated and the antibody titers were determined by ELISA. Values are the mean ± standard deviation of 5 mice/group of ELISA which was run in triplicates.

**Specificity of antibodies**

Competitive ELISA was used to assess the ability of the anti-hapten antibodies to bind to heroin, 6-acetylmorphine and morphine. . Sera from mice immunized with 6-PrOxyHap were inhibited by heroin, 6-acetymorphine, morphine and codeine (Fig 6A, Table 1). The $IC_{50}$ values varied considerably



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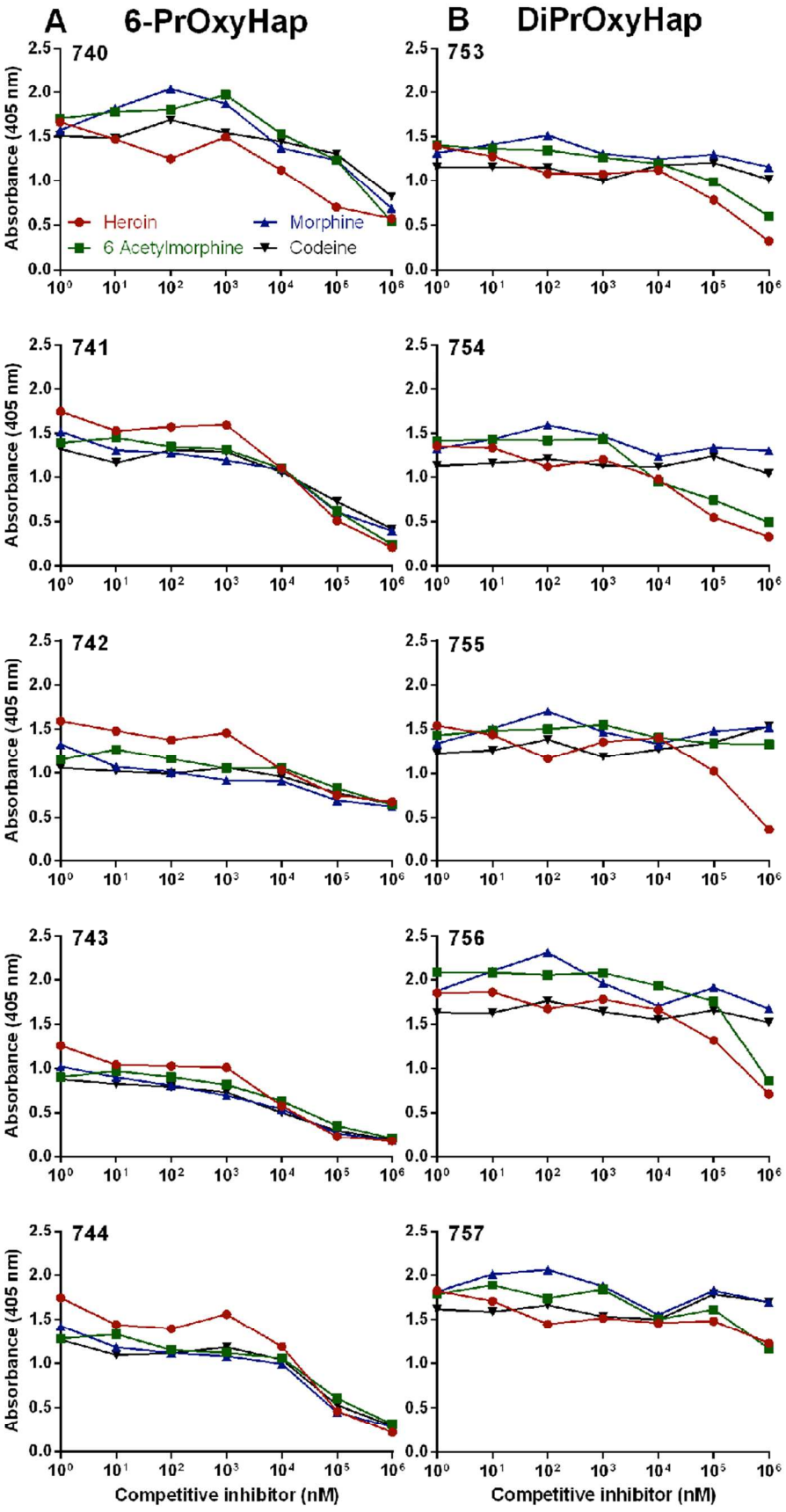


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**Fig. 6** Competitive inhibition in an ELISA of the antibodies with heroin, 6-acetylmorphine, morphine and codeine. Sera were from week 9. Values are the mean of triplicate determinations with standard deviation 10% or less. Each graph represents the serum from a different animal. $IC_{50}$ were calculated and are shown in Table 1.

among the 5 sera. Morphine and codeine did not compete for the binding of sera from DiPrOxyHap immunized animals to DiPrOxyHap (Fig. 6B, Table 1). Binding was blocked by heroin in sera from 4 of the 5 animals and by 6-acetymorphine in 3 of the 5 animals. The data in Fig 6 and Table 1 suggest that 6-PrOxyHap induces antibodies that bind more effectively to heroin, 6-acetymorphine, morphine and codeine than antibodies from DiPrOxyHap immunized mice. As was previously shown by Matyas et al.[12] antibodies from DiAmHap immunized animals had very high affinity binding to DiAmHap. Due to this high affinity binding, heroin, 6-acetymorphine and morphine did not compete for binding to DiAmHap coated plates. However, when a morphine based hapten, MorHap, was used as the coating antigen in the ELISA, heroin, 6-acetymorphine, and morphine effectively competed the binding of the DiAmHap antisera to the MorHap coated plate. This demonstrated that the competition ELISA itself has a fundamental flaw in the ability of the free drug, heroin, 6-acetylmophorine and morphine, to compete effectively and is highly dependent upon the affinity of the antibodies for the coating hapten. Consequently, the values for $IC_{50}$ >1,000 in table 1 for DiPrOxyHap antisera might represent high affinity antibodies to DiPrOxyHap that cannot be competed by 6-acetylmophine, morphine and codeine.

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**Table 1** Competition of sera from animals immunized with the hapten conjugated to tetanus toxoid with opiates for the binding to the hapten conjugated to bovine serum albumin[a]

| Immunogen/ Capture antigen | Mouse number | Inhibition concentration ($IC_{50}$)[b] (μM) | | | |
|---|---|---|---|---|---|
| | | Heroin | 6-Acetylmorphine | Morphine | Codeine |
| 6-PrOxyHap | | | | | |
| | 740 | 17 | 140 | 62 | 268 |
| | 741 | 19 | 43 | 28 | 50 |
| | 742 | 90 | 520 | 500 | 530 |
| | 743 | 8 | 15 | 7 | 8 |
| | 744 | 28 | 50 | 28 | 49 |
| DiPrOxyHap | | | | | |
| | 753 | 130 | 125 | >1000 | >1000 |
| | 754 | 24 | 100 | >1000 | >1000 |
| | 755 | 266 | >1000 | >1000 | >1000 |
| | 756 | 158 | 470 | >1000 | >1000 |
| | 757 | >1000 | >1000 | >1000 | >1000 |

[a]Sera were from animals bled 3 weeks after the last immunization.

[b]$IC_{50}$ values were calculated from the data shown in Fig. 6 using nonlinear regression one-site-fit log$IC_{50}$ model.

**Inhibition of heroin antinociception**

The immunized mice and unimmunized control mice were tested for antinociceptive effects of injected heroin using the hot plate assay. Six out of 8 mice immunized with 6-PrOxyHap and 5 out of 8 mice immunized with DiOxyHap had a reduced effect of heroin (Fig. 7). The % MPE for mice immunized with 6-PrOxyHap was significantly reduced compared to unimmunized control animals. The data for antinociceptive effects of immunization with DiAmHap has recently been reported[12] with a % MPE of 54.7.

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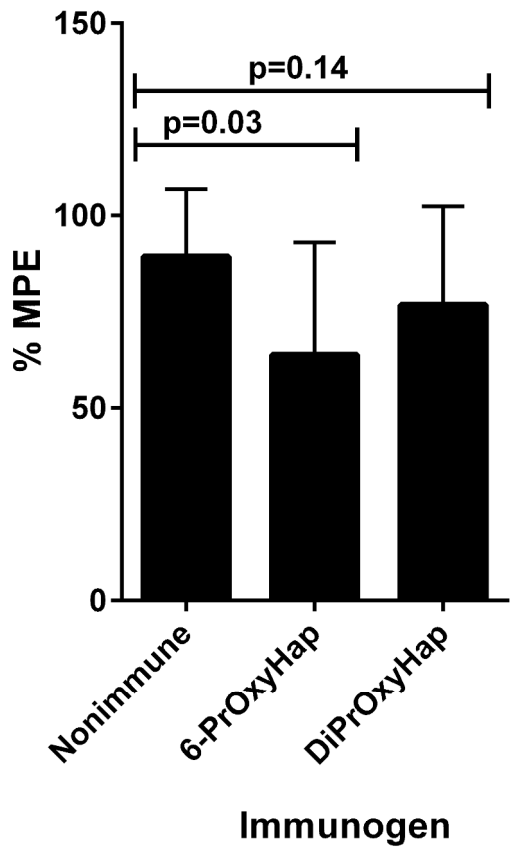


**Fig. 7** Heroin-induced antinociception of immunized mice. Animals (8/group) were challenged 9 weeks after primary immunization with heroin HCl (0.75 mg/kg) by the subcutaneous route. Measurements for the hot plate nociception assay were taken prior to and 20 min after heroin injection. Values are % MPE ± standard deviation. Significance of % MPE of immunized animals as compared to unimmunized control animals were calculated using an unpaired T-test. % MPE for DiAmHap immunized animals was shown in Matyas et al.[12] at 54.6 (p=0.0025 compared to control animals).

**Comparison with other haptens**

Coupling of heroin or morphine to protein carriers has been primarily done at positions C3 or C6 or at the bridge nitrogen atom. Several laboratories have coupled morphine at the C6 position by various coupling strategies.[2, 7, 9, 33-36] This strategy has induced antibodies that primarily react with morphine

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and 6-acetylmorphine, but less so with heroin. However, challenge of animals that were immunized with C6 coupled opiates blocked heroin in both nociception[14, 33] and self-administration models in rodents[9, 36] presumably due to rapid degradation of heroin to 6-acetylmorphine in the blood following injection.

Janda et al., have coupled heroin at the bridge nitrogen atom producing a hapten called Her or HerHap.[7, 37, 38] Immunization with this hapten induced antibodies that cross-reacted with heroin, 6-acetylmorphine and morphine and protected animals from heroin challenge. As described by those authors, HerHap is a "dynamic" hapten, degrading by deacetylation at the C3 and the C6 position after immunization, thereby inducing antibodies to heroin and its primary degradation products, 6-acetylmorphine and morphine.[7, 37] Although this degradation after immunization seems advantageous, this same degradation of HerHap would be expected in a vialed vaccine, causing a short shelf life of the vialed vaccine. Morphine coupled at the bridge nitrogen was also tested as a vaccine; it abrogated the effect of morphine challenge, but not heroin.[37] The same group also tested the N-linked hapten with an acetamide at the C3 position.[5] Immunization with the 3-acetamide hapten induced antibodies that failed to react effectively with 6-acetylmorphine, leading the authors to conclude that it would not be an effective vaccine formulation. The DiAmHap and DiPrOxHap that we have synthesized are novel heroin hapten designs that are chemically stable with the linker at the bridge nitrogen. Immunization with DiAmHap produced both higher titer antibodies than DiPrOxyHap and protected mice against heroin challenge in the hot-plate assay, where DiPrOxyHap immunization did not. DiAmHap represents a unique stable N-linked heroin hapten that induced protective immunity and, consequently, is a viable alternative to HerHap as potential heroin vaccine and likely to be more useful because of its inherent stability.

Very few studies have been reported using 3-position coupled opiates. We have previously shown that immunization with 6-AcMorHap induced significantly lower antibodies titers (400,000)



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than immunization with MorHap, HerHap or DiAmHap.[2,12] Taken together with the antibody titers obtained by immunization with 6-PrOxyHap reported here, the data suggest that 3-position conjugation is not optimal for the induction of high antibody titer following immunization. However, immunization with 6-PrOxyHap coupled to tetanus toxoid and mixed with liposomal MPLA did protect against heroin challenge in a nociception assay and, consequently, warrants further investigation and optimization.

It is difficult to compare immunization studies with the various haptens used by other laboratories because different animal models, immunization schedules and doses, adjuvants, carrier and ELISA conditions have been used. In addition, antibody titers are calculated differently in each laboratory. However, it is clear that immunization with carriers containing morphine coupled at the C6 position or surrogate heroin haptens coupled at the bridge nitrogen induced high titer antibodies that can protect against heroin challenge. Both 6-PrOxyHap and DiAmHap represent new potential haptens that are candidates for further studies as heroin vaccines.

**Conclusions**

A reproducible synthetic sequence for installation of a 3-amino group on 4,5-epoxymorphinan scaffolds was developed. A homologation at the C6 position was studied and an efficient route was developed for construction of the C6α side chain; the two key elements in the syntheses. Using these methodologies, three haptens were prepared, PrOxyHap **1** and DiPrOxyHap **2,** and DiAmHap **3**. Mice immunized with DiAmHap had higher titer antibodies than those immunized with 6-PrOxyHap or DiPrOxyHap and the antibodies from DiAmHap immunized mice appear to have a higher affinity than those from animals immunized with the other haptens. In addition, DiAmHap immunized mice were better protected from the antinociceptive effects of heroin. Taken together, these data suggest that DiAmHap is a better candidate for a heroin vaccine than 6-PrOxyHap and DiPrOxyHap.

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## Experimental Section

### Synthesis General Methods

All reactions were performed in glassware containing a Teflon coated stir bar. All reagents were obtained from commercial sources and used without further purifications. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton nuclear magnetic resonance ($^1$H NMR, 400 or 500 MHz) and carbon nuclear magnetic resonance ($^{13}$C NMR, 100 or 125 MHz) spectra were recorded on a Bruker DMX 400 and 500 wide-bore spectrometer in $CDCl_3$ (unless otherwise noted) with the values given in ppm and *J* (Hz) assignments of 1H resonance coupling. For $^1$H NMR spectra ($CDCl_3$), the residual solvent peak was used as the reference (7.26 ppm) while the central solvent peak was used as the $^{13}$C NMR reference (77.0 ppm in $CDCl_3$). The high-resolution electrospray ionization (ESI) mass spectra were obtained on a Waters LCT Premier time-off light (TOF) mass spectrometer. Thin-layer chromatography (TLC) was performed on 0.25 mm Analtech GHLF silica gel and used to determine the completion of the reaction (solvent system: $CHCl_3$/MeOH /$NH_4OH$ (19:0.9:0.1 or 9:0.9:0.1)) depending on the polarity of the compounds. Gas chromatography (GC) was performed on an Agilent Technologies 6850 Series system equipped with Agilent Technologies 7683B series injector and Agilent Technologies 5975C VL MSD Triple-Axis detector. Flash column chromatography was performed with Bodman silica gel LC 60 A. Elemental analyses were performed by Micro-Analysis, Inc, Wilmington, DE, and were within 0.4% for C, H, and N.

### (4*S*,4a*R*,7a*R*,12b*R*)-3-Methyl-7-oxo-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-9-yl trifluoromethanesulfonate (5)

To a suspension of hydromorphone hydrochloride **3** (1.6 g, 5 mmol) and $Et_3N$ (1.26 g, 1.73 mL, 12.5 mmol) in anhydrous $CH_2Cl_2$ (50 mL) under argon was added *N*-phenyl-

bis(trifluoromethanesulfonimide) (2.14 g, 6 mmol) in small portions at room temperature. The reaction mixture was stirred overnight and the solvent was evaporated. The residue was dissolved into 2 M HCl and the resulting solution was washed with ether. The aqueous layer was then made basic with $NH_4OH$ to pH 9 and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. Filtration and evaporation afforded a light yellow solid **5** (2.0 g, 95.8%), which was used directly without further purification. $[\alpha]^{20}_D$ -179.3° (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.99 (d, $J$ = 8.5 Hz, 1H), 6.73 (d, $J$ = 8.5 Hz, 1H), 4.78 (s, 1H), 3.21 (m, 1H), 3.07 (d, $J$ = 19.0 Hz, 1H), 2.60 (m, 2H), 2.43 (s, 3H), 2.39 (m, 4H), 2.11 (m, 2H), 1.86 (m, 1H), 1.76 (m, 1H), 1.21 (m, 1H); $^{13}$C NMR ($CDCl_3$ + $CD_3OD$, 100 MHz): $\delta$ 205.4, 148.2, 132.5, 131.4, 128.5, 123.1, 120.6, 91.7, 59.8, 47.0, 46.0, 42.0, 40.1, 39.4, 33.6, 24.8, 21.0; ESI-MS $(M+H)^+$ 418.1; HRMS (*m/z*): [$ES^+$, $M+H]^+$ calcd for $C_{18}H_{19}NO_5F_3S$ 418.0936, found 418.0929.

**(4*S*,4a*R*,7a*R*,12b*R*)-3-Methyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (6)**

A flask charged with triflate **5** (2.0 g, 4.78 mmol), palladium acetate (0.11 g, 0.48 mmol), formic acid (0.552 g, 12.0 mmol), triethylamine (1.46 g, 2.0 mL, 14.4 mmol), triphenylphosphine (0.252 g, 0.96 mmol) and DMF (20 mL) was evacuated and backfilled with argon. The reaction mixture was stirred at 60 $^o$C overnight. The solvent was evaporated and the reminder was treated with water. The resulting solution was basified with $NH_4OH$ to pH 9 and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:MeOH:$NH_4OH$, 95:4.5:0.5) to yield a gray solid **6** (1.32 g, quant.). Mp 233-234 $^o$C (mp 241.5-245 $^o$C[17]); $[\alpha]^{20}_D$ -268.8° (*c* 0.9, $CHCl_3$) ($[\alpha]^{21}_D$ -250.6° (*c* 1.06, MeOH)).[17] $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.04 (t, $J$ = 8.0 Hz, 1H), 6.74 (d, $J$ = 8.0 Hz, 1H), 6.68 (d, $J$ = 8.0 Hz, 1H), 4.61 (s, 1H), 3.18 (m, 1H), 3.05 (d, $J$ = 18.5 Hz, 1H), 2.56 (m, 2H), 2.41 (s, 3H), 2.35 (m, 3H),



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2.16 (td, $J$ = 12.5, 3.5 Hz, 1H), 2.05 (td, $J$ = 12.5, 5.0 Hz, 1H), 1.83 (m, 1H), 1.75 (d, $J$ = 12.5 Hz, 1H), 1.23 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.5, 157.8, 134.5, 128.9, 125.6, 119.1, 107.9, 90.8, 59.2, 46.8, 46.1, 42.9, 42.8, 40.3, 35.6, 25.4, 20.6; ESI-MS 270.1 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{17}H_{20}NO_2$ 270.1494, found 270.1496;

**(4*R*,4a*R*,7a*R*,12b*S*)-11-Bromo-3-methyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (7a)**

To a solution of compound **6** (0.33 g, 1.22 mmol) in 0.1 M $H_2SO_4$ (20 mL) was added *N*-bromosuccinimide (NBS, 0.238 g, 1.34 mmol) in small portions at room temperature. The reaction mixture was stirred for 1h and basified with $NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. Filtration and evaporation afforded a yellow solid **7a** (408 mg, 96.2%), which was pure enough to be used directly. Mp 259-260 $^o$C; $[\alpha]^{20}_D$ -198.9° (*c* 1.1, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.25 (d, $J$ = 8.5 Hz, 1H), 6.69 (d, $J$ = 8.0 Hz, 1H), 4.64 (s, 1H), 3.25 (m, 1H), 2.97 (d, $J$ = 19.0 Hz, 1H), 2.57 (m, 2H), 2.43 (s, 3H), 2.40 (m, 3H), 2.20 (dd, $J$ = 19.5, 5.5 Hz, 1H), 2.09 (m, 1H), 1.85 (m, 1H), 1.77 (d, $J$ = 10.5 Hz, 1H), 1.20 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 207.7, 157.0, 134.2, 131.8, 128.0, 113.3, 110.1, 91.1, 59.1, 46.7, 46.5, 42.9, 42.4, 40.2, 35.5, 25.3, 22.1; ESI-MS 348.1 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{17}H_{19}NO_2Br$ 348.0599, found, 348.0588.

**(4*R*,4a*R*,7a*R*,12b*S*)-11-Chloro-3-methyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (7b)**

To a solution of compound **6** (1.3 g, 4.79 mmol) in 0.1 M $H_2SO_4$ (80 mL) was added *N*-chlorosuccinimide (NCS, 1.28 g, 9.58 mmol) in small portions at room temperature and the reaction solution was stirred 3 h at 90 $^o$C. After cooling to 0 $^o$C in ice-water bath, the solution was basified with



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$NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the residue was purified by flash chromatography ($CHCl_3$:MeOH:$NH_4OH$, 95:4.5:0.5) to afforded a light yellow solid **7b** (1.13 g, 77.7%). Mp 248-249 $^oC$; $[\alpha]^{20}_D$ -246.8° (*c* 0.8, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.07 (d, *J* = 8.5 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 5.64 (s, 1H), 3.24 (m, 1H), 3.02 (d, *J* = 19.0 Hz, 1H), 2.57 (m, 2H), 2.43 (s, 3H), 2.38 (m, 2H), 2.23 (dd, *J* = 19.5, 5.5 Hz, 1H), 2.08 (m, 2H), 1.84 (m, 1H), 1.76 (d, *J* = 11.5 Hz, 1H), 1.19 (m, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 207.8, 156.4, 132.4, 128.7, 127.7, 124.0, 109.4, 91.2, 58.8, 46.6, 46.5, 42.8, 42.4, 40.2, 35.5, 25.3, 19.7; ESI-MS 304.1 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{17}H_{19}NO_2Cl$ 304.1104, found 304.1099

**(4*R*,4a*R*,7a*R*,12b*S*)-11-Chloro-3-methyl-9-nitro-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-one (8b)**

To a solution of chloride **7b** (0.664 g, 2.2 mmol) in TFA (25 mL) was added $NaNO_2$ (0.612 g, 8.8 mmol) in small portions and the resulting brown solution was stirred for 20 h to give an orange solution. The reaction solution was alkalized with $NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:MeOH:$NH_4OH$, 95:4.5:0.5) to yield an orange foam **13b** (0.5 g, 66.0%). $[\alpha]^{20}_D$ -123° (*c* 0.2, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.96 (s, 1H), 5.00 (s, 1H), 3.31 (m, 1H), 3.09 (d, *J* = 20.0 Hz, 1H), 2.68 (dd, *J* = 9.5, 2.5 Hz, 1H), 2.61 (dd, *J* = 7.5, 4.0 Hz, 1H), 2.48 (m, 5H), 2.29 (dd, *J* = 20.0, 5.5 Hz, 1H), 2.16 (td, *J* = 12.5, 5.0 Hz, 1H), 2.04 (dd, *J* = 12.0, 3.5 Hz, 1H), 1.93 (dd, *J* = 13.5, 4.0 Hz, 1H), 1.82 (d, *J* = 12.0 Hz, 1H), 1.19 (m, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 204.8, 151.2, 140.0, 131.90, 131.86, 124.9, 124.3, 93.2, 58.4, 47.1, 46.1, 42.9, 42.1, 40.0, 35.4, 25.6, 20.5; ESI-MS 349.1 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{17}H_{18}N_2O_4Cl$ 349.0955, found 349.0953.

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Single-crystal X-ray diffraction data on compound **8b**, CCDC deposition number 975660. Data were collected using MoKα radiation and a Bruker APEX II CCD area detector. The crystal was prepared for data collection by coating with high viscosity microscope oil. The oil-coated crystal was mounted on a micro-mesh mount (MiteGen, Inc.) and transferred to the diffractometer and a data set collected at 150°K. The 0.51 x 0.43 x 0.01 $mm^3$crystal was orthorhombic in space group P $2_12_12_1$, with unit cell dimensions a = 7.3581(12), b = 9.1360(16), and c = 23.005(4) Å. Data was 99.9% complete to 26.48° θ (~ 0.80 Å) with an average redundancy of 7.34. The final anisotropic full matrix least-squares refinement on $F^2$ with 218 variables converged at R1 = 2.84%, for the observed data and wR2 = 7.02% for all data. The structure was solved by direct methods and refined by full-matrix least squares on F2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI). Corrections were applied for Lorentz, polarization, and absorption effects. Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C-H distance set at 0.96 Å. Complete information on data collection and refinement is available in the supplemental material.

**(*E*)-1-((4*R*,4a*R*,7a*S*,12b*S*)-11-Chloro-3-methyl-9-nitro-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-ylidene)propan-2-one (9a) and (*Z*)-1-((4*R*,4a*R*,7a*S*,12b*S*)-11-chloro-3-methyl-9-nitro-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-ylidene)propan-2-one (9b)**

To a solution of ketone **8b** (0.3 g, 0.86 mmol), dimethyl (2-oxopropylphosphonate) (214 mg, 1.29 mmol) in a mixed solvent (THF:EtOH:$H_2O$, 4:4:1, 18 mL) was added KOH (72 mg, 1.29 mmol) and the resulting suspension was stirred for 3h at ambient temperature. The reaction was quenched with saturated $NH_4Cl$ and the solvent was distilled off. The residue was dissolved into water and basified



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with 28% ammonium hydroxide to pH 9.5 and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:MeOH:$NH_4OH$, 95:4.5:0.5) to afford compound **9a** (126.0 mg, 35.9%) as a bright yellow foam, and **9b** (50 mg, 14.9%) as a light yellow foam.

**9a**: $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.90 (s, 1H), 6.55 (s, 1H), 5.15 (s, 1H), 3.75 (d, $J$ = 14.5 Hz, 1H), 3.20 (s, 1H), 3.03 (m, 1H), 2.57 (d, $J$ = 7.5 Hz, 1H), 2.45 (d, $J$ = 12.5 Hz, 1H), 2.38 (m, 4H), 2.23 (dd, $J$ = 19.5, 5.5 Hz, 1H), 2.16 (s, 3H), 2.00 (m, 1H), 1.71 (m, 3H), 0.79 (m, 1H); ESI-MS 389.1 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{22}N_2O_4Cl$ 389.1268, found 389.1255.

**9b**: Mp 212.1-213.2 $^oC$; $[\alpha]^{20}_D$ -99.4° (*c* 0.7, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.92 (s, 1H), 5.86 (s, 1H), 5.79 (s, 1H), 3.21 (m, 1H), 3.10 (d, $J$ = 20.0 Hz, 1H), 2.56 (d, $J$ = 12.5 Hz, 1H), 2.40 (s, 3H), 2.32 (m, 2H), 2.28 (s, 3H), 2.18 (m, 2H), 2.01 (m, 2H), 1.73 (d, $J$ = 11.5 Hz, 1H), 1.64 (m, 1H), 0.91 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 201.2, 150.7, 144.9, 140.4, 133.7, 131.4, 129.8, 124.2, 123.7, 90.1, 58.6, 46.1, 43.9, 42.8, 41.3, 35.5, 31.8, 30.9, 24.3, 20.4; ESI-MS 389.1 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{22}N_2O_4Cl$ 389.1268, found 389.1258.

**1-((4*R*,4a*R*,7a*S*,12b*S*)-9-Amino-11-chloro-3-methyl-2,3,4,4a,5,7a-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one (10) and (*E*)-1-((4*R*,4a*R*,7a*S*,12b*S*)-9-amino-11-chloro-3-methyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-ylidene)propan-2-one (11)**

A suspension of compound **9a** (26 mg, 0.067 mmol), Fe (15 mg, 0.268 mmol), $NH_4Cl$ (21 mg, 0.402 mmol) in a mixed solvent (EtOH and $H_2O$ 3/1 v/v, 2 mL) was refluxed under argon for 2h. After cooling to room temperature, the mixture was filtered through a pad of celite and washed with MeOH. The filtrate was concentrated *in vacuo* and the residue was dissolved in water and basified with 28%



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ammonium hydroxide, and then extracted with $CHCl_3$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. Filtration and evaporation yielded a yellow oil containing compounds **10** and **11**, (21 mg, 87.5%) whose polarities are too similar to be separated completely. Only a part of the less polar compound **10** was obtained pure as a yellow solid. $[\alpha]^{20}_D$ -234.1° (*c* 2.0, $CHCl_3$,); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.53 (s, 1H), 5.69 (d, $J$ = 5.5 Hz, 1H), 4.84 (s, 1H), 3.43 (s, 2H, $NH_2$), 3.24 (d, $J$ = 15.5 Hz, 1H), 3.17 (m, 1H), 3.06 (d, $J$ = 15.5 Hz, 1H), 2.90 (d, $J$ = 19.0 Hz, 1H), 2.54 (d, $J$ = 12.0, 4.5 Hz, 1H), 2.42 (s, 3H), 2.39 (m, 1H), 2.26 (m, 2H), 2.12 (s, 3H), 1.99 (m, 1H), 1.91 (td, $J$ = 12.5, 5.0 Hz, 1H), 1.76 (d, $J$ = 12.5 Hz, 1H), 1.55 (m, 1H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 207.3, 143.0, 131.4, 130.5, 129.7, 129.5, 123.4, 122.0, 115.8, 90.1, 58.9, 49.8, 46.8, 43.1, 41.8, 38.3, 35.2, 29.4, 25.0, 19.3; ESI-MS 359.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{24}N_2O_2Cl$ 359.1526, found 359.1515.

**11**: yellow foam; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.53 (s, 1H), 6.49 (d, $J$ = 4.8 Hz, 1H), 4.81 (s, 1H), 3.75 (m, 1H), 3.52 (s, 2H, $NH_2$), 3.14 (m, 1H), 2.92 (d, $J$ = 15.2 Hz, 1H), 2.53 (m, 1H), 2.39 (m, 4H), 2.12 (m, 5H), 1.94 (m, 1H), 1.72 (m, 2H), 1.63 (m, 2H), 0.89 (m, 1H); ESI-MS 359.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{24}N_2O_2Cl$ 359.1526, found 359.1523.

**(4*R*,4a*R*,7a*S*,12b*S*)-3-Methyl-7-methylene-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzo-furo [3,2-*e*]isoquinoline (12a)**

A flask charged with methyltriphenylphosphonium bromide (2.14 g, 6 mmol) was evacuated and backfilled with argon. Dry THF (20 mL) and a solution of *n*-BuLi (1.6 M in THF, 2.8 mL) were added successively. The resulting orange solution was stirred for 3 h at room temperature. A solution of **6** (0.54 g, 2 mmol) in THF (5 mL) was added and the stirring was continued overnight. The solvent was evaporated and the residue was treated with saturated $NH_4Cl$ and 28% $NH_4OH$. The suspension was extracted with $CH_2Cl_2$ and the combined extracts were washed with brine and dried over anhydrous



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$Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 95:4.5:0.5) to yield **12a** (0.5 g, 93.8%) as an orange oil, which crystallized from hexane and EtOAc (10:1) as white needle-like crystals. Mp 277-279 $^oC$; $[\alpha]^{20}_D$ -154.6° (*c* 0.5. $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.03 (t, *J* = 8.0 Hz, 1H), 6.64 (t, *J* = 8.0 Hz, 2H), 5.20 (d, *J* = 1.6 Hz, 1H), 4.86 (s, 1H), 4.80 (d, *J* = 2.0 Hz, 1H), 3.07 (m, 1H), 3.01 (d, *J* = 18.8 Hz, 1H), 2.53 (dd, *J* = 12.0, 4.8 Hz, 1H), 2.40 (d, 3H), 2.33 (m, 2H), 2.24 (dt, *J* = 14.0, 3.2 Hz, 1H), 2.17 (td, *J* = 12.0, 4.0 Hz, 1H), 1.98 (t, *J* = 13.2 Hz, 1H), 1.90 (td, *J* = 12.4, 5.2 Hz, 1H), 1.71 (m, 1H), 1.57 (m, 1H), 0.92 (qd, *J* = 12.8, 2.8 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 157.6, 145.7, 135.0, 128.1, 127.8, 118.3, 111.2, 107.6, 89.4, 59.7, 47.5, 43.64, 43.57, 42.9, 35.4, 32.6, 26.5, 20.7; ESI-MS 268.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{18}H_{22}NO$ 268.1701, found 268.1711.

**((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-Methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)methanol (13a)**

To a solution of **12a** (0.93 g, 3.48 mmol) in anhydrous THF (30 mL) at 0 $^oC$ under argon was added a solution of $BH_3$-DMS (1 M in THF, 15.5 mL) and the solution was stirred for 17 h at room temperature. The reaction mixture was cooled to 0 $^oC$ again. EtOH (12 mL), NaOH (3 M, 6 mL) and $H_2O_2$ (35%wt, 4.3 mL) were added successively and the stirring was continued for 3 days. The solvent was evaporated and the residue was treated with saturated $NH_4Cl$ and 28% $NH_4OH$. The suspension was extracted with $CH_2Cl_2$ and the combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 90:9:1) to afford ((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)methanol **13a** (0.424 g, 42.7%) as a white foam, which crystallized from MeOH/$Et_2O$ as white crystals. Mp 177~178 $^oC$; $[\alpha]^{20}_D$ -235.2° (*c* 1.0, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.02 (t, *J* = 7.6 Hz, 1H), 6.62 (d, *J* = 7.6 Hz,



Organic & Biomolecular Chemistry Accepted Manuscript

1H), 6.53 (d, *J* = 7.6 Hz, 1H), 4.77 (d, *J* = 3.2 Hz, 1H), 3.68 (m, 1H), 3.54 (m, 1H), 3.10 (m, 1H), 2.99 (d, *J* = 18.8 Hz, 1H), 2.50 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.42 (dd, *J* = 18.8, 6.4 Hz, 1H), 2.38 (s, 3H), 2.26 (m, 2H), 1.93 (m, 2H), 1.70 (m, 1H), 1.59 (m, 1H), 1.25 (m, 1H), 0.82 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.4, 136.0, 128.9, 128.4, 118.2, 105.5, 90.4, 63.7, 59.8, 46.4, 43.2, 41.5, 39.5, 37.8, 36.9, 20.64, 20.59, 18.0; ESI-MS 286.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{18}H_{24}NO_2$ 286.1807, found 286.1813. Anal. Found: C, 75.15; H, 7.91, N, 5.06. Calcd for $C_{18}H_{23}NO_2 \bullet 0.1H_2O$: C, 75.28; H, 8.14; N, 4.88%.

Single-crystal X-ray diffraction data on compound **13a**, CCDC deposition number 954929. Data were collected using CuKα radiation and a Bruker Platinum-135 CCD area detector. The crystal was prepared for data collection by coating with high viscosity microscope oil. The oil-coated crystal was mounted on a micro-mesh mount (Mitergen, Inc.) and transferred to the diffractometer and a data set collected at 100°K. The 0.258 x 0.184 x 0.058 $mm^3$crystal was orthorhombic in space group P $2_12_12_1$, with unit cell dimensions a = 6.8411(3), b = 12.9417(6), and c = 16.7006(12) Å. Data was 74.1% complete to 67.85° θ (~ 0.83 Å) with an average redundancy of 5.12. The final anisotropic full matrix least-squares refinement on $F^2$ with 192 variables converged at R1 = 5.40%, for the observed data and wR2 = 13.20% for all data. The structure was solved by direct methods and refined by full-matrix least squares on F2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI). Corrections were applied for Lorentz, polarization, and absorption effects. Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C-H distance set at 0.96 Å. Complete information on data collection and refinement is available in the supplemental material.

Organic & Biomolecular Chemistry Accepted Manuscript

**((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-Methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)methyl methanesulfonate (14a)**

To a solution of **13a** (60 mg, 0.21 mmol) in $CH_2Cl_2$ (5 mL) was added $Et_3N$ (58 μL, 0.42 mmol) and MsCl (24 uL, 0.32 mmol) at 0 $^oC$ under argon. The solution was stirred for 2 h at room temperature. The reaction was quenched by addition of a solution of saturated $NaHCO_3$ and the organic layer was separated and washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 95:4.5:.5) to give **14a** (0.08 g, quant.) as a clear oil, which solidified on standing. $[\alpha]^{20}_D$ -297.3° (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.03 (t, *J* = 7.6 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.53 (d, *J* = 7.6 Hz, 1H), 4.67 (d, *J* = 3.6 Hz, 1H), 4.29 (t, *J* = 8.8 Hz, 1H), 4.04 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.11 (m, 1H), 3.04 (s, 3H), 3.00 (d, *J* = 19.2 Hz, 1H), 2.50 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.42 (dd, *J* = 19.2, 6.4 Hz, 1H), 2.38 (s, 3H), 2.26 (m, 2H), 2.13 (m, 1H), 1.90 (td, *J* = 12.4, 5.2 Hz, 1H), 1.70 (m, 1H), 1.61 (m, 1H), 1.28 (m, 1H), 0.78 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 159.4, 136.2, 128.54, 128.51, 118.4, 105.6, 87.9, 70.4, 59.7, 46.3, 43.2, 41.6, 37.9, 37.2, 37.1, 36.5, 20.6, 20.2, 17.5; ESI-MS 364.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{19}H_{26}NO_4S$ 364.1583, found 364.1574.

**2-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-Methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)acetonitrile (15a)**

To a solution of **14a** (2.15 g, 5.9 mmol) was added 18-crown-6 (0.1 g), NaI (0.18 g, 1.2 mmol) and KCN (1.16 g, 17.8 mmol) successively. The resulting solution was heated to 100$^oC$ for 40 h under argon. The solvent was evaporated and the reminder was treated with water and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the residue was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 95:4.5:0.5) to **15a** (1.5 g, 86.2%) as a yellow oil. $[\alpha]^{20}_D$ -214.4° (*c* 1.0, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.05



Organic & Biomolecular Chemistry Accepted Manuscript

(t, *J* = 8.0 Hz, 1H), 6.66 (d, *J* = 7.0 Hz, 1H), 6.56 (d, *J* = 7.5 Hz, 1H), 4.60 (s, 1H), 3.13 (s, 1H), 3.02 (d, *J* = 19.0 Hz, 1H), 2.53 (m, 2H), 2.42 (m, 1H), 2.40 (s, 3H), 2.28 (m, 3H), 2.07 (m, 1H), 1.92 (m, 1H), 1.72 (d, *J* = 12.5 Hz, 1H), 1.65 (m, 1H), 1.42 (m, 1H), 0.86 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 159.4, 136.2, 128.7, 128.4, 119.0, 118.6, 105.8, 89.5, 70.7, 59.8, 46.4, 43.3, 42.0, 38.0, 36.7, 35.1, 21.2, 20.6, 19.5; ESI-MS 295.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{19}H_{22}N_2O$ 295.1810, found 295.1810.

**1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-Methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one (16a)**

To a solution of compound **15a** (80 mg, 0.27 mmol) was added a solution of MeLi (1.6 M in $Et_2O$, 0.84 mL, 1.35 mmol) under argon at 0 $^oC$. The solution was warmed to room temperature gradually and stirred for 12h. A solution of HCl (2 M, 2 mL) was added and the stirring was continued for 2 h. The solvent was evaporated and the reminder was basified with 28% $NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the residue was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$,95:4.5:0.5) to give **16a** (62 mg, 73.8%) as a yellow oil. $[\alpha]^{20}_D$ -203.2° (*c* 1.3, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.04 (t, *J* = 8.0 Hz, 1H), 6.63 (d, *J* = 7.6 Hz, 1H), 6.54 (d, *J* = 7.6 Hz, 1H), 4.57 (d, *J* = 2.8 Hz, 1H), 3.10 (dd, *J* = 6.0, 1.2 Hz, 1H), 3.00 (d, *J* = 18.8 Hz, 1H), 2.71 (m, 1H), 2.50 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.42 (m, 1H), 2.40 (s, 3H), 2.28 (m, 4H), 2.16 (s, 1H), 1.92 (td, *J* = 12.4, 5.2 Hz, 1H), 1.64 (m, 2H), 1.22 (m, 1H), 0.77 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.1, 159.6, 136.2, 129.0, 128.3, 118.0, 105.3, 91.2, 59.9, 46.4, 44.8, 43.2, 41.6, 37.9, 36.5, 32.8, 30.7, 21.6, 20.8, 20.6; ESI-MS 312.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{26}NO_2$ 312.1964, found 312.1955.

**(4*R*,4a*R*,7a*S*,12b*S*)-9-Methoxy-3-methyl-7-methylene-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline (12b)**



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A flask charged with methyltriphenylphosphonium bromide (7.1 g, 20 mmol) was evacuated and backfilled with argon. THF (100 mL) was added followed by addition of a solution of *n*-BuLi (2.5 M in THF, 10 mL) at 0 $^{o}$C (ice-water bath). The resulting orange solution was stirred at room temperature for 3 h. A solution of hydrocodone **4** (3.0 g, 10 mmol) in THF (50 mL) was added dropwise and the stirring was continued for 16 h. The reaction was quenched by water and the solvent was evaporated and the residue was dissolved in $CH_2Cl_2$ (100 mL). The solution was washed with water and brine and dried over anhydrous $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 19:0.9:0.1) to afford **12b** (2.57 g, 86.5%) as a pale-white solid. Mp 115-117 $^{o}$C (mp 127-129 $^{o}$C)[25]; $[\alpha]^{20}_{D}$ -142.0° (*c* 0.8, $CHCl_3$) ($[\alpha]^{21}_{D}$ -123° (c 0.87, $CHCl_3$)).[25] $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.68 (d, *J* = 8.4 Hz, 1H), 6.59 (d, *J* = 8.0 Hz, 1H), 5.26 (d, *J* = 1.2 Hz, 1H), 4.89 (s, 1H), 4.80 (d, *J* = 2.0 Hz, 1H), 3.87 (s, 3H), 3.06 (m, 1H), 2.97 (d, *J* = 18.4 Hz, 1H), 2.54 (dd, *J* = 12.0, 3.6 Hz, 1H), 2.40 (s, 3H), 2.28 (m, 3H), 2.18 (td, *J* = 12.4, 4.0 Hz, 1H), 1.98 (m, 1H), 1.90 (td, *J* = 12.4, 4.8 Hz, 1H), 1.74 (m, 1H), 1.56 (m, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 145.6, 145.1, 143.1, 129.4, 126.9, 118.9, 113.9, 111.1, 90.0, 59.8, 56.8, 47.6, 44.3, 43.4, 42.9, 35.4, 32.5, 26.4, 20.1; ESI-MS, 298.2 $(M+1)^{+}$; HRMS calcd for ($C_{19}H_{24}NO_2$) 298.1807, found 298.1799.

**((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-Methoxy-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)methanol (13b)**

To a solution of **13b** (2.27 g, 7.6 mmol) in THF (50 mL) was added a solution of $BH_3$-THF (1 M, 23 mL) at 0 $^{o}$C under nitrogen, and the resulting solution was stirred at room temperature overnight. The reaction was not complete (by TLC) and 5 mL of $BH_3$-THF was added and the stirring was continued for an additional 24 h. The reaction solution was cooled to 0 $^{o}$C again and EtOH (30 mL) was added followed by the addition of a solution of NaOH (3 M, 7.7 mL) and $H_2O_2$ (35%, 4.7 mL). The stirring was continued for 16 h and the solvent was evaporated and the residue was treated with saturated

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$NH_4Cl$ and $NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 9:0.9:0.1) to afford **13b** (1.67 g, 69.6%) as a white solid. Mp 169-170 $^oC$; $[\alpha]^{20}_D$ -179.0° (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$Hz) $\delta$ 6.69 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 8.0 Hz, 1H), 4.82 (dd, *J* = 3.2, 0.8 Hz, 1H), 3.83 (s, 3H), 3.72 (m, 1H), 3.56 (m, 1H), 3.08 (m, 1H), 2.96 (d, *J* = 18.4 Hz, 1H), 2.48 (dd, *J* = 12.0, 4.0 Hz, 1H), 2.38 (m, 4H), 2.25 (m, 2H), 1.90 (m, 2H), 1.72 (m, 1H), 1.58 (m, 1H), 1.28 (m, 1H), 0.88 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 146.9, 141.5, 130.4, 128.0, 118.7, 113.5, 91.5, 63.9, 59.9, 56.6, 46.5, 43.2, 42.3, 39.5, 37.9, 37.0, 20.6, 20.0, 18.3; ESI-MS, 316.2 $(M+1)^+$; HRMS calcd for ($C_{19}H_{26}NO_3$) 316.1913, found 316.1917.

**((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-Methoxy-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)methyl methanesulfonate (14b)**

To a solution of ((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-methoxy-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)methanol **13b** (1.67 g, 5.3 mmol) in $CH_2Cl_2$ (50 mL) and $Et_3N$ (1.47 mL, 10.6 mmol) was added dropwise a solution of MsCl (0.62 mL, 8 mmol) in $CH_2Cl_2$ (5 mL) at 0 $^oC$ and the solution was stirred for 2 h at room temperature. The solution was washed with saturated $NaHCO_3$ and brine and dried over $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 19:0.9:0.1) to yield **14b** (1.99 g, 95.7%) as a yellow oil. $[\alpha]^{20}_D$ -214.6° (*c* 1.0, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.70 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 8.0 Hz, 1H), 4.82 (d, *J* = 3.2 Hz, 1H), 4.33 (dd, *J* = 9.6, 8.0 Hz, 1H), 4.06 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.83 (s, 3H), 3.10 (dd, *J* = 6.0, 2.4 Hz, 1H), 3.04 (s, 3H), 2.96 (d, *J* = 18.4 Hz, 1H), 2.49 (dd, *J* = 12.4, 4.4 Hz, 1H), 2.39 (m, 4H), 2.26 (m, 2H), 2.14 (m 1H), 1.90 (td, *J* = 12.4, 5.2 Hz, 1H), 1.71 (m, 1H), 1.62 (m, 1H), 1.32 (m, 1H), 0.83 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 146.8, 141.5, 130.0, 128.0, 119.0,

Organic & Biomolecular Chemistry Accepted Manuscript

113.8, 88.8, 70.5, 59.8, 56.6, 46.3, 43.2, 42.3, 37.8, 37.2, 37.1, 36.5, 20.2, 19.9, 17.8; ESI-MS, 394.2 $(M+1)^+$; HRMS calcd for ($C_{20}H_{28}NO_5S$) 394.1688, found 394.1679.

**2-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-Methoxy-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methano-benzofuro[3,2-*e*]isoquinolin-7-yl)acetonitrile (15b)**

To a solution of **14b** (1.99 g, 5.1 mmol) in DMF (80 mL) was added KCN (0.665 g, 10.2 mmol), NaI (0.15 g, 1.02 mmol) and 18-crown-6 (0.1 g) and the mixture was evacuated and backfilled with nitrogen and heated to 100 $^oC$ for 30 h. The solvent was evaporated and the residue was dissolved in $CH_2Cl_2$ (100 mL). The solution was washed with water and brine and dried over $Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 19:0.9:0.1) to yield **20b** (1.37 g, 83.5%) as a brown oil. $[\alpha]^{20}_D$ = -183.1° ($CHCl_3$, *c* 0.9); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.70 (d, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 4.62 (dd, *J* = 4.4, 1.2 Hz, 1H), 3.84 (s, 3H), 3.09 (dd, *J* = 6.0, 2.8 Hz, 1H), 2.96 (d, *J* = 18.4 Hz, 1H), 2.57 (dd, *J* = 16.8, 6.4 Hz, 1H), 2.48 (dd, *J* = 12.0, 4.0 Hz, 1H), 2.37 (m, 4H), 2.25 (m, 3H), 2.11 (m 1H), 1.89 (td, *J* = 12.0, 5.2 Hz, 1H), 1.71 (m, 1H), 1.61 (m, 1H), 1.45 (m, 1H), 0.92 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 146.6, 141.5, 129.8, 127.9, 119.2, 119.0, 113.8, 90.2, 59.6, 56.6, 46.3, 43.2, 42.7, 37.9, 36.7, 35.0, 21.1, 20.4, 19.9, 19.3; ESI-MS, 325.2 $(M+1)^+$; HRMS calcd for ($C_{20}H_{25}N_2O_2$) 325.1916, found 325.1925.

**1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-Methoxy-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methano-benzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one (16b)**

To a solution of **15b** (86 mg, 0.3 mmol) in $Et_2O$ (10 mL) was added a solution of methylmagnesium bromide (3M in $Et_2O$, 0.35 mL) at 0 $^oC$. The solution was stirred at room temperature for 12h. GC indicated only 50% conversion. 1 mL of methylmagnesium bromide was added and the stirring was



Organic & Biomolecular Chemistry Accepted Manuscript

continued for additional 6h. THF (0.5 mL) was added and a viscous precipitate appeared. The stir bar was stuck and the stirring stopped. After 12h, the reaction mixture was cooled to 0 $^{o}$C and HCl (2 M, 3 mL) was added. The solution was stirred for 1h and the solvent was evaporated. The residue was basified with 28% $NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with water and brine and dried over $Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 19:0.9:0.1) to yield **16b** (45 mg, 50.5%) as yellow oil. $[\alpha]^{20}_{D}$ = -194.2° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.69 (d, *J* = 8.0 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 1H), 4.61 (d, *J* = 3.2 Hz, 1H), 3.85 (s, 3H), 3.09 (dd, *J* = 6.0, 2.8 Hz, 1H), 2.95 (d, *J* = 18.8 Hz, 1H), 2.75 (dd, *J* = 17.6, 5.6 Hz, 1H), 2.50 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.39 (m, 5H), 2.28 (m, 3H), 2.13 (s, 3H), 1.92 (td, *J* = 12.8, 5.2 Hz, 1H), 1.67 (m, 1H), 1.61 (m, 1H), 1.21 (m, 1H), 0.81 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.0, 147.0, 141.4, 130.4, 128.0, 118.5, 113.5, 92.1, 59.9, 56.6, 46.4, 44.8, 43.1, 42.3, 37.8, 36.4, 32.5, 30.7, 21.5, 20.8, 20.0; ESI-MS, 342.2 $(M+1)^+$; HRMS calcd for ($C_{21}H_{28}NO_3$) 342.2069, found 342.2066;.

**1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-11-Chloro-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methano-benzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one (17)**

To a solution of **16a** (0.16 g, 0.52 mmol) in 0.1 M $H_2SO_4$ was added *N*-chlorosuccinimide (82 mg, 0.62 mmol) in small portions at room temperature. The solution was heated to 90 $^{o}$C for 3 h and then cooled to 0 $^{o}$C and basified with 28% $NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the residue was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 95:4.5:0.5) to give **17** (140 mg, 77.8%) as a yellow oil. $[\alpha]^{20}_{D}$ = -215.2° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.04 (d, *J* = 8.4 Hz, 1H), 6.50 (d, *J* = 8.4 Hz, 1H), 4.60 (d, *J* = 2.8 Hz, 1H), 3.14 (dd, *J* = 6.0, 2.4 Hz, 1H), 2.90 (d, *J* = 19.2 Hz, 1H), 2.69 (m, 1H), 2.49 (dd, *J* = 12.4, 4.8 Hz, 1H), 2.39 (m, 4H), 2.25 (m, 4H), 2.15 (s, 3H), 1.90 (td, *J* = 12.4,



Organic & Biomolecular Chemistry Accepted Manuscript

4.8 Hz, 1H), 1.64 (m, 2H), 1.22 (m, 1H), 0.74 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.3, 158.1, 133.8, 131.1, 128.1, 122.8, 106.9, 91.8, 59.5, 46.1, 44.7, 43.3, 42.0, 37.8, 36.1, 32.5, 30.9, 21.4, 20.6, 19.8; ESI-MS 346.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{25}NO_2Cl$ 346.1574, found 346.1572.

**1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-11-Chloro-3-methyl-9-nitro-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one (18)**

To a solution of **17** (0.58 g, 1.7 mmol) in TFA (17 mL) was added $NaNO_2$ (0.24 g, 3.4 mmol) in small portion at 0 $^oC$. The orange solution was stirred for 1.5 h and poured into an ice-cooled solution of ammonia and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the residue was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 95:4.5:0.5) to give **18** (0.55 g, 82.8%) as a yellow foam. $[\alpha]^{20}_D$ = -58.9° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.93 (s, 1H), 4.94 (d, *J* = 2.4 Hz, 1H), 3.20 (dd, *J* = 6.0, 2.8 Hz, 1H), 2.94 (d, *J* = 20.0 Hz, 1H), 2.79 (dd, *J* = 20.0, 8.8 Hz, 1H), 2.54 (dd, *J* = 12.0, 4.0 Hz, 1H), 2.39 (m, 7H), 2.19 (s, 3H), 2.12 (td, *J* = 12.4, 3.2 Hz, 1H), 1.98 (td, *J* = 12.0, 4.8 Hz, 1H), 1.72 (m, 2H), 1.28 (m, 1H), 0.73 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 207.5, 153.2, 141.4, 135.6, 130.0, 123.7, 123.3, 95.2, 59.0, 45.6, 44.2, 43.1, 42.1, 37.3, 35.5, 32.4, 30.8, 21.1, 20.5, 20.4; ESI-MS 391.1 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{24}N_2O_4Cl$ 391.1425, found 391.1425;

**1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-Amino-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one (19)**

A glass bomb charged with the base **18** (1.22 g, 3.1 mmol), 10% Pd/C (0.1 g) and 30% AcOH (100 mL) was evacuated and backfilled with $H_2$. The mixture was hydrogenated at 50 psi overnight. The solvent was evaporated and the residue was basified with ammonia and extracted with $CH_2Cl_2$. After filtration and evaporation, the residue was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$,



Organic & Biomolecular Chemistry Accepted Manuscript

90:9:1) to give **19** (0.68 g, 68%) as an off-white solid. $[\alpha]^{20}_{D}$ = -162.8° ($CHCl_3$, *c* 0.9); mp 141-143 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.50 (q, *J* = 8.0 Hz, 2H), 4.60 (d, *J* = 3.2 Hz, 1H), 3.40 (brs, 2H, $NH_2$), 3.08 (d, *J* = 6.0, 2.8 Hz, 1H), 2.93 (d, *J* = 18.4 Hz, 1H), 2.70 (m, 1H), 2.49 (dd, *J* = 12.0, 4.8 Hz, 1H), 2.39 (s, 3H), 2.30 (m, 5H), 2.24 (s, 3H), 1.90 (td, *J* = 12.4, 4.8 Hz, 1H), 1.67 (dd, *J* = 12.4, 1.6 Hz, 1H), 1.60 (m, 1H), 1.20 (m, 1H), 0.82 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.2, 146.7, 129.1, 126.8, 125.9, 118.7, 116.4, 91.5, 60.0, 46.6, 44.9, 43.2, 42.4, 37.8, 36.8, 32.8, 30.7, 21.8, 20.8, 20.0; ESI-MS 327.2 $(M+1)^+$; HRMS ($ES^+$) calcd for $C_{20}H_{27}N_2O_2$, 327.2703, found 327.2082.

**(*N*-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-Methyl-7-(2-oxopropyl)-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-9-yl)-3-(tritylthio)propanamide) (1)**

To a solution of 1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-amino-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one **19** (0.65 g, 2 mmol) in $CH_2Cl_2$ (20 mL) under Ar at 0 °C was added $Et_3N$ (0.83 mL, 6 mmol), 3-(tritylthio)propanoic acid (1.04 g, 3 mmol) and O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU, 1.28 g, 4 mmol) and the resulting solution was stirred at ambient temperature overnight. The reaction solution was cooled to 0 °C again and saturated $NaHCO_3$ (20 mL) was added. The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$. The combined extracts were washed with brined and dried over anhydrous $Na_2SO_4$. After filtration and evaporation, the crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$,19:0.9:0.1) to give 6-PrOxyHap **1** (1.15 g, 87.8%) as a white powder after freeze-drying with *t*-BuOH. $[\alpha]^{20}_{D}$ = -88.1° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.76 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 6H), 7.28 (t, *J* = 8.0 Hz, 6H), 7.21 (t, *J* = 7.2 Hz, 3H), 6.61 (d, *J* = 8.4 Hz, 1H), 4.66 (d, *J* = 3.2 Hz, 1H), 3.08 (dd, *J* = 6.0, 2.8 Hz, 1H), 2.96 (d, *J* = 18.8 Hz, 1H), 2.60 (m, 3H), 2.48 (dd, *J* = 11.6, 4.0 Hz, 1H), 2.39 (m, 4H), 2.25 (m, 4H), 2.15 (m, 2H), 2.08 (s, 3H), 1.91 (td, *J* = 12.4, 5.2 Hz, 1H), 1.62 (m, 2H), 1.18 (m, 1H), 0.77 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$)

Organic & Biomolecular Chemistry Accepted Manuscript

$\delta$ 208.0, 170.0, 148.7, 144.7 (3), 131.8, 129.6 (6), 129.0, 128.0 (6), 126.8 (3), 121.0, 118.6, 118.2, 92.7, 67.0, 59.8, 46.3, 44.8, 43.2, 42.4, 37.8, 36.6, 36.5, 32.7, 30.7, 27.7, 21.6, 20.7, 20.3; ESI-MS, 657.3 $(M+1)^+$; HRMS calcd for ($C_{42}H_{45}N_2O_3S$) 657.3151, found 657.3138. Anal. Found: C, 74.52; H, 7.32; N, 4.03. Calcd for $C_{42}H_{44}N_2O_3S$•0.5$^t$BuOH•0.75$H_2O$: C, 74.70; H, 7.20; N 3.96%.

**1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-Hydroxy-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)propan-2-one (20)**

To a solution of $BBr_3$ (1 M in $CH_2Cl_2$, 8.4 mL) in $CHCl_3$ (30 mL) under nitrogen at 0 $^oC$ was added dropwise a solution of **16b** (0.48 g, 1.4 mmol) in $CHCl_3$ (10 mL). The solution was stirred at room temperature for 15 min and cooled to -78 $^oC$ and the reaction was quenched by $CH_3OH$. The solution was warmed to room temperature and stirred for 30 min and the solvent was evaporated. The residue was basified with $NH_4OH$ and extracted with $CHCl_3$/MeOH (20/1, V/V). The combined extracts were dried over $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 9:0.9:0.1) to afford **20** (0.39 g, 84.8%) as a white solid. $[\alpha]^{20}_D$ = -133.7° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.65 (d, *J* = 8.0 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 4.63 (d, *J* = 3.2 Hz, 1H), 3.13 (dd, *J* = 6.0, 2.8 Hz, 1H), 2.95 (d, *J* = 18.8 Hz, 1H), 2.70 (m, 1H), 2.55 (dd, *J* = 12.4, 5.2 Hz, 1H), 2.42 (m, 4H), 2.31 (m, 4H), 2.14 (s, 3H), 1.96 (td, *J* = 17.6, 5.2 Hz, 1H), 1.66 (m, 1H), 1.59 (m, 1H), 1.21 (m, 1H), 0.82 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.2, 145.8, 137.5, 130.1, 126.9, 119.0, 116.8, 92.0, 59.8, 46.4, 44.9, 43.0, 42.5, 37.6, 36.4, 32.7, 30.6, 21.8, 20.8, 20.1; ESI-MS, 328.2 $(M+1)^+$; HRMS calcd for ($C_{20}H_{26}NO_3$) 328.1913, found 328.1925.

**(4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-methyl-7-(2-oxopropyl)-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methano-benzofuro[3,2-*e*]isoquinolin-9-yl trifluoromethanesulfonate (21)**

Organic & Biomolecular Chemistry Accepted Manuscript

A mixture of **20** (0.39 g, 1.19 mmol), $Et_3N$ (0.5 mL, 3.6 mmol) and *N*-phenyl-bis(trifluoromethanesulfonimide) (0.86 g, 2.4 mmol) in $CH_2Cl_2$ was stirred overnight at room temperature. The solvent was evaporated and the residue was treated with HCl (2 M, 10 mL). The aqueous solution was washed with $Et_2O$ and basified with 28% $NH_4OH$ and extracted with $CH_2Cl_2$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$,19:0.9:0.1) to afford **21** (0.5 g, 91.4%) as yellow oil. $[\alpha]^{20}_D$ = -207.1° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.92 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 4.77 (dd, *J* = 3.6, 1.2 Hz, 1H), 3.11 (dd, *J* = 6.0, 2.8 Hz, 1H), 2.97 (d, *J* = 19.2 Hz, 1H), 2.78 (dd, *J* = 18.0, 7.2 Hz, 1H), 2.50 (dd, *J* = 12.0, 4.0 Hz, 1H), 2.40 (m, 5H), 2.25 (m, 3H), 2.15 (s, 3H), 1.94 (td, *J* = 13.6, 5.2 Hz, 1H), 1.66 (m, 2H), 1.20 (m, 1H), 0.74 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.1, 150.1, 136.3, 133.0, 129.7, 121.3, 120.3 and 117.1 ($J_{C,F}$ = 318.7), 119.3, 94.1, 59.6, 46.0, 44.0, 43.1, 42.6, 37.4, 35.7, 32.5, 30.8, 20.8, 20.52, 20.47; ESI-MS, 460.1 $(M+1)^+$; HRMS calcd for ($C_{21}H_{25}NO_5SF_3$) 460.1406, found 460.1400.

**1-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-9-Allyl-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzo-furo[3,2-*e*]isoquinolin-7-yl)propan-2-one (22)**

To a round flask charged with a mixture of **21** (0.2 g, 0.44 mmol), anhydrous LiCl (155 mg, 3.6 mmol), $PPh_3$ (69 mg, 0.26 mmol), $PdCl_2(PPh_3)_2$ (37 mg, 0.05 mmol) and DMF (20 mL) was added allyltributyltin (0.54 mL, 1.7 mmol) and the mixture was heated to 120 °C for 16 h under nitrogen. The solvent was evaporated and the water and $CH_2Cl_2$ were added. The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 19:0.9:0.1) to afford **22** (108 mg, 70.7%) as a yellow oil. $[\alpha]^{20}_D$ = -193.1° ($CHCl_3$, *c* 1.9); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.87 (d, *J* = 7.6 Hz, 1H), 6.59 (d, *J* = 8.0 Hz, 1H), 5.96 (m, 1H), 5.06 (m, 1H), 5.02 (m, 1H), 4.58 (d, *J* = 2.8 Hz, 1H), 3.31 (m, 2H), 3.11 (dd, *J* = 8.8, 2.8 Hz,

Organic & Biomolecular Chemistry Accepted Manuscript

1H), 2.98 (d, *J* = 18.8 Hz, 1H), 2.71 (m, 1H), 2.52 (dd, *J* = 11.6, 4.0 Hz, 1H), 2.43 (dd, *J* = 20.8, 6.4 Hz, 1H), 2.41 (s, 3H), 2.28 (m, 4H), 2.15 (s, 3H), 1.94 (td, *J* = 12.4, 5.2 Hz, 1H), 1.67 (m, 1H), 1.60 (m, 1H), 1.16 (m, 1H), 0.80 (m, 1H), 0.66 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.2, 157.5, 136.8, 133.7, 129.0, 128.7, 118.3, 117.6, 115.2, 91.0, 60.0, 46.4, 44.7, 43.1, 41.8, 37.6, 36.2, 33.8, 32.8, 30.8, 21.6, 20.8, 20.5; ESI-MS, 352.2 $(M+1)^+$; HRMS calcd for ($C_{23}H_{30}NO_2$) 352.2277, found 352.2276.

**1,1'-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-3-Methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline-7,9-diyl)bis(propan-2-one) (23)**

To a solution of **22** (30 mg, 0.085 mmol) in THF (3 mL) and $H_2O$ (1 mL) was added mercuric acetate (54 mg, 0.17 mmol) was stirred at room temperature for 18 h. The reaction mixture was transferred to a solution of LiCl (12 mg, 0.28 mmol), $PdCl_2$ (25 mg, 0.14 mmol) and $CuCl_2$ (57 mg, 0.42 mmol) in THF (3 mL) and $H_2O$ (1 mL) and the mixture was heated to 55 $^oC$ for 4 h. The solvent was evaporated and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 19:0.9:0.1) to afford **23** (23 mg, 71.9%) as a yellow oil. $[\alpha]^{20}_D$ = -255.9° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.85 (d, *J* = 7.6 Hz, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 4.59 (d, *J* = 2.8 Hz, 1H), 3.69 (d, *J* = 15.6 Hz, 1H), 3.53 (d, *J* = 16.0 Hz, 1H), 3.09 (dd, *J* = 6.4, 2.8 Hz, 1H), 2.98 (d, *J* = 18.8 Hz, 1H), 2.66 (m, 1H), 2.49 (dd, *J* = 11.6, 4.0 Hz, 1H), 2.41 (dd, *J* = 20.8, 6.4 Hz, 1H), 2.39 (s, 3H), 2.26 (m, 4H), 2.17 (s, 3H), 2.16 (s, 3H), 1.92 (td, *J* = 12.8, 5.2 Hz, 1H), 1.63 (m, 2H), 1.18 (m, 1H), 0.76 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.2, 206.2, 157.9, 135.2, 129.7, 129.0, 118.6, 112.2, 91.6, 59.8, 46.3, 44.8, 44.6, 43.2, 42.0, 37.8, 36.2, 32.7, 30.8, 29.4, 21.3, 20.8, 20.5; ESI-MS, 368.2 $(M+1)^+$; HRMS calcd for ($C_{23}H_{30}NO_3$) 368.2226, found 368.2240;

Organic & Biomolecular Chemistry Accepted Manuscript

**1,1'-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-2,3,4,4a,5,6,7,7a-Octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]iso-quinoline-7,9-diyl)bis(propan-2-one) (24)**

To a solution **23** (23 mg, 0.06 mmol) in 1,2-dichloroethane (5 mL) was added $NaHCO_3$ (42 mg, 0.5 mmol) and chloroethylchloroformate (14 μL, 0.13 mmol) successively and the mixture was refluxed for 1 h under nitrogen. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in MeOH (5 mL) and the solution was stirred for 1h. The solvent was removed and basified with 28% $NH_4OH$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over anhydrous $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 9:0.9:0.1) to afford **24** (17 mg, 77.3%) as a yellow oil. $[\alpha]^{20}_D$ = -270.8° ($CHCl_3$, *c* 0.9); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.86 (d, *J* = 8.0 Hz, 1H), 6.62 (d, *J* = 7.6 Hz, 1H), 4.56 (d, *J* = 2.4 Hz, 1H), 3.70 (d, *J* = 15.6 Hz, 1H), 3.54 (d, *J* = 16.0 Hz, 1H), 3.36 (dd, *J* = 6.8, 3.2 Hz, 1H), 2.99 (dd, *J* = 18.8, 6.8 Hz, 1H), 2.75 (m, 4H), 2.25 (m, 2H), 2.17 (s, 3H), 2.16 (s, 3H), 1.85 (m, 1H), 1.81 (s, 1H, br), 1.77 (td, *J* = 12.8, 5.6 Hz, 1H), 1.62 (m, 2H), 1.18 (m, 1H), 0.74 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.2, 206.2, 158.0, 135.3, 129.8, 129.1, 118.6, 112.2, 91.9, 52.7, 44.8, 44.6, 42.9, 38.6, 38.4, 37.1, 32.6, 31.5, 30.8, 29.4, 21.5, 21.2; ESI-MS, 354.2 $(M+1)^+$; HRMS calcd for ($C_{22}H_{28}NO_3$) 354.2069, found 354.2059.

***N*-(4-((4*R*,4a*R*,7*R*,7a*S*,12b*S*)-7,9-bis(2-oxopropyl)-4,4a,5,6,7,7a-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-3(2*H*)-yl)butyl)-3-(tritylthio)propanamide (2)**

To a solution of **24** (17 mg, 0.048 mmol) in DMF (3 mL) was added $K_2CO_3$ (33 mg, 0.24 mmol) and *tert*-butyl (4-bromobutyl)carbamate (18 mg, 0.072 mmol) and the mixture was heated to 50 °C for 24 h. The solvent was evaporated and the residue was treated with $H_2O$ and extracted with $CH_2Cl_2$. The combined extracts were washed with brine and dried over $Na_2SO_4$. After filtration, the filtrate was concentrated to around 5 mL and TFA (1 mL) was added. The solution was stirred at room temperature

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for 1.5 h and concentrated. The residue was dried under high vacuum for 0.5 h and dissolved in $CH_2Cl_2$ (5 mL). 2,5-Dioxopyrrolidin-1-yl-3-(tritylthio)propanoate (43 mg, 0.096 mmol) and $Et_3N$ (33 μL, 0.24 mmol) were added and the solution was stirred for 16 h. The reaction solution was washed with water and brine and dried over $Na_2SO_4$. The crude product was purified by flash chromatography ($CHCl_3$:$CH_3OH$:$NH_4OH$, 19:0.9:0.1) to afford hapten **2** (27 mg, 74.6% over 3 steps) as a yellow foam after freeze-drying with *t*-BuOH. $[\alpha]^{20}_D$ = -110.2° ($CHCl_3$, *c* 1.0); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.41 (d, *J* = 8.0 Hz, 6H), 7.27 (t, *J* = 8.0 Hz, 6H), 7.20 (t, *J* = 7.2 Hz, 3H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.60 (d, *J* = 7.6 Hz, 1H), 5.72 (brs, 1H, NH), 4.59 (d, *J* = 2.8 Hz, 1H), 3.70 (d, *J* = 15.6 Hz, 1H), 3.53 (d, *J* = 15.6 Hz, 1H), 3.20 (m, 2H), 3.13 (dd, *J* = 6.0, 2.4 Hz, 1H), 2.89 (d, *J* = 18.8 Hz, 1H), 2.67 (m, 1H), 2.47 (m, 6H), 2.24 (m, 4H), 2.17 (s, 3H), 2.16 (s, 3H), 2.05 (t, *J* = 7.2 Hz, 2H), 1.87 (td, *J* = 12.4, 4.8 Hz, 1H), 1.60 (m, 2H), 1.50 (m, 4H), 1.17 (m, 1H), 0.74 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 208.1, 206.2, 170.8, 157.9, 144.7 (3), 135.1, 129.8 (6), 129.6, 129.1, 127.9 (6), 126.7 (3), 118.6, 112.2, 91.5, 66.8, 57.7, 54.4, 44.8, 44.6, 44.5, 42.6, 39.4, 37.8, ,36.1, 35.8, 32.7, 30.8, 29.4, 27.8, 27.3, 25.0, 21.4, 21.3, 20.9; ESI-MS, 755.4 $(M+1)^+$; HRMS calcd for ($C_{48}H_{55}N_2O_4S$) 755.3883, found 755.3861. Anal. Found: C, 74.43; H, 7.18; N, 3.65. Calcd for $C_{48}H_{54}N_2O_4S•H_2O$: C, 74.58; H, 7.30; N, 3.62%.

**(4*S*,4a*R*,7a*R*,12b*R*)-*N*-Benzyl-3-methyl-2,3,4,4a,5,6-hexahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7(7a*H*)-imine (25)**

A toluene solution (100 mL) containing the intermediate 3-deoxy-dihydromorphinone (**6**, 1.20 g, 4.46 mmol), benzylamine (550 mg, 5.13 mmol), and a trace of *p*-toluenesulfonic acid was refluxed for 10 h, using a Dean-Stark trap for azeotropic removal of $H_2O$. The mixture was then concentrated (20 mL) at 1 atm, and a solution of $NaBH_4$, (70 mg, 1.75 mmol) in absolute EtOH (40 mL) was added. After being stirred under argon for 3 h, the resulting solution was diluted with $H_2O$ and concentrated to remove most of the EtOH. Further dilution with $H_2O$, basification ($NH_4OH$), extraction ($CHCl_3$), and



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concentration of the organic phase afforded crude **25** (1.50 g) that was used without purification to prepare **26**.

**(4*S*,4a*R*,7*S*,7a*R*,12b*R*)-*N*-Benzyl-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-amine (26)**

The crude amine **25** was dissolved in $CH_2Cl_2$ (100 mL) and 4 drops of acetic acid and $NaB(OAc)_3H$ (945 mg, 4.46 mmol) were added. The resulting mixture was stirred at room temperature for 24 h. After filtration through celite the solution was concentrated in vacuo. The residue was purified through silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 90:10:1) to give **26** (884 mg, 55% yield in two steps) as a pale syrup. $[\alpha]_D^{20}$ -197.4° (*c* 1.64, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.36 (t, 2H, *J* = 7.0 Hz), 7.33 (t, 2H, *J* = 7.0 Hz), 7.24-7.28 (m, 1H), 7.06 (t, 1H, *J* = 7.5 Hz), 6.65 (d, 1H, *J* = 7.5 Hz), 6.60 (d, 1H, *J* = 7.5 Hz), 5.30 (s, 1H), 4.76 (d, 1H, *J* = 3.5 Hz), 3.89 (dd, 2H, *J* = 37.5, 13.0 Hz), 3.10 (dd, 1H, *J* = 6.0, 2.5 Hz), 3.02 (d, 1H, *J* = 18.5 Hz), 2.81 (dt, 1H, *J* = 12.0, 3.5 Hz), 2.51 (dd, 1H, *J* = 12.0, 5.0 Hz), 2.43 (dd, 1H, *J* = 14.5, 6.5 Hz), 2.40 (s, 3H), 2.28 (td, 1H, *J* = 12.5, 3.5 Hz), 2.17-2.23 (m, 1H), 1.91 (td, 1H, *J* = 12.5, 5.0 Hz), 1.73 (d, 1H, *J* = 12.5 Hz), 1.60-1.68 (m, 1H), 1.54 (t, 1H, *J* = 9.0 Hz), 0.78-0.92 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 159.6, 140.5, 136.0, 129.1, 128.5, 128.2, 127.0, 118.2, 105.8, 89.4, 59.7, 54.1, 50.6, 46.2, 43.2, 41.9, 38.2, 36.7, 20.0, 21.0, 20.6; HRMS ($ES^+$) calcd for $C_{24}H_{29}N_2O$ 361.2280, found: 361.2281.

**(4*S*,4a*R*,7*S*,7a*R*,12b*R*)-3-Methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-amine (27)**

10% (w/w) Pd/C (400 mg) and concd HCl (0.5 mL) were added to a solution of **26** (860 mg, 2.39 mmol) in MeOH (80 mL) and the mixture was stirred under $H_2$ (45 psi) for 6 days at room temperature. The reaction mixture was filtered through a Celite pad, and concentrated in vacuo. Purification by silica



Organic & Biomolecular Chemistry Accepted Manuscript

gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 90:10:1) gave **27** (548 mg, 85%) as a solid. An analytical sample of the hydrobromide salt of **27** was prepared. Amine **27** was dissolved in methanol and treated with 33% HBr in AcOH to pH ~1. The solution was concentrated, dissolved in MeOH and concentrated again to give a yellow solid. The solid was dissolved in a minimal volume of boiling 1:1 $H_2O$:acetone and additional acetone was added until a yellow oil began to separate from the solution. Methanol was added to the boiling solution to dissolve the oil, followed by acetone to initiate precipitation of a white solid. The solution was cooled to room temperature then to 2-8 °C for 16 h. The solids were collected via vacuum filtration and rinsed with cold 1:1 acetone-MeOH to give a light yellow solid. For recrystallization, the solid was dissolved in boiling MeOH and allowed to cool to give **27**□HBr as a white solid. Mp 305 °C (dec); $[\alpha]_D^{20}$ □225.4° (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.04 (t, 1H, *J* = 7.5 Hz), 6.63 (d, 1H, *J* = 7.5 Hz), 6.58 (d, 1H, *J* = 7.5 Hz), 6.51 (d, 1H, *J* = 4.5 Hz), 3.08 (dd, 1H, *J* = 6.0, 2.5 Hz), 2.99-3.04 (m, 1H), 3.00 (d, 1H, *J* = 18.5 Hz), 2.48 (dd, 1H, *J* = 12.0, 4.5 Hz), 2.40 (dd, 1H, *J* = 14.5, 6.5 Hz), 2.39 (s, 3H), 2.24 (qd, 2H, *J* = 12.0, 3.5 Hz), 1.89 (td, 1H, *J* = 12.5, 5.0 Hz), 1.69 (dd, 1H, *J* = 12.5, 2.0 Hz), 1.54-1.63 (m, 1H), 1.34-1.41 (m, 1H), 1.25 (br, 2H), 0.99-1.09 (m, 1H), 0.82-0.91 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 159.6, 135.8, 129.0, 128.5, 118.3, 105.8, 91.9, 59.8, 49.4, 46.4, 43.2, 42.0, 38.0, 38.0, 26.3, 20.8, 20.6; HRMS ($ES^+$) calcd for $C_{17}H_{23}N_2O$ 271.1810, found: 271.1813. Anal. Found: C, 45.81; H, 5.99; N, 6.09. Calcd for $C_{17}H_{22}N_2O$•2HBr•0.6MeOH•0.5 $H_2O$: C, 45.91; H, 6.00; N, 6.08%.

Single-crystal X-ray diffraction data on compound **27**, CCDC deposition number 955757. The 0.263 x 0.221 x 0.167 $mm^3$ data crystal was monoclinic in space group P $2_1$, with unit cell dimensions a = 8.1091(2), b = 11.2247(3), c = 11.0425(4) Å, and β = 106.116(1)° . The data was 98.4% complete to 29.14° θ (~ 0.73 Å) with an average redundancy of 4.11. The final anisotropic full matrix least-squares refinement on $F^2$ with 96 variables and one constraint converged at R1 = 3.93%, for the observed data and wR2 = 10.51% for all data. The absolute configuration was determined from the x-

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ray data (Flack parameter = -0.002(8). The structure was solved by direct methods and refined by full-matrix least squares on F2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI). Corrections were applied for Lorentz, polarization, and absorption effects. Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C-H distance set at 0.96 Å. Complete information on data collection and refinement is available in the supplemental material.

**(4*S*,4a*R*,7*S*,7a*R*,12b*R*)-11-Chloro-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-amine (28)**

With stirring at room temperature, 3-deoxy-6-amino-dihydromorphine (**27**, 520 mg 1.93 mmol), and *N*-chlorosuccinimide (514 mg, 3.85 mmol) were added to 0.1 N $H_2SO_4$ (40 mL), and the mixture was heated at 90 °C for 3 h. After cooling to room temperature, NaOH (300 mg, 7.5 mmol) was added to the reaction mixture.[18] The crude product was extracted with $CHCl_3$ and the organic solution was dried over $MgSO_4$. After concentration in vacuo, the residue was purified by silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 90:10:1) to give **28** (453 mg, 77% yield) as a solid. Mp 187-192 °C; $[\alpha]_D^{20}$ -220.4° (*c* 1.0, $CHCl_3$);$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.06 (dd, 1H, $J$ = 8.5, 0.5 Hz), 6.55 (d, 1H, $J$ = 8.5 Hz), 4.54 (d, 1H, $J$ = 4.0 Hz), 3.13 (dd, 1H, $J$ = 6.0, 2.5 Hz), 3.00-3.06 (m, 1H), 2.92 (dd, 1H, $J$ = 19.0 Hz), 2.49 (dd, 1H, $J$ = 18.5, 4.5 Hz), 2.40 (s, 3H), 2.33 (dd, 1H, $J$ = 19.5, 6.0 Hz), 2.17-2.25 (m, 2H), 1.89 (td, 1H, $J$ = 12.5, 5.0 Hz), 1.68 (dd, 1H, $J$ = 12.5, 2.0 Hz), 1.56-1.64 (m, 1H), 1.34-1.42 (m, 1H), 1.25 (br, 2H), 0.97-1.08 (m, 1H), 0.81-0.90 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 158.2, 133.5, 131.1, 128.3, 123.0, 107.3, 92.6, 59.5, 49.3, 46.2, 43.2, 42.4, 37.9, 37.5, 26.2, 20.5, 19.9; HRMS ($ES^+$) calcd for $C_{17}H_{22}ClN_2$ 305.1421, found 305.1428.

Organic & Biomolecular Chemistry Accepted Manuscript

**_N_-((4_S_,4a_R_,7_S_,7a_R_,12b_R_)-11-Chloro-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1_H_-4,12-methanobenzofuro[3,2-_e_]isoquinolin-7-yl)acetamide (29)**

To a stirred mixture of **28** (420 mg, 1.38 mmol), triethylamine (418 mg, 4.13 mmol) and DMAP (10 mg) in dry $CH_2Cl_2$ (20 mL) at 0 °C was added acetic anhydride (287 mg, 2.76 mmol). The resulting solution was slowly warmed to room temperature and stirred overnight. After concentration in vacuo the residue was purified by silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 90:10:1) to give **29** (426 mg, 89% yield) as a syrup. $[\alpha]_D^{20}$ -162.5° (*c* 1.0, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.09 (d, 1H, *J* = 8.5 Hz), 6.57 (d, 1H, *J* = 8.5 Hz), 5.82 (d, 1H, *J* = 8.5 Hz), 4.60 (d, 1H, *J* = 4.0 Hz), 4.15-4.22 (m, 1H), 3.26 (dd, 1H, *J* = 6.0, 3.0 Hz), 2.92 (d, 1H, *J* = 19.5 Hz), 2.62 (dd, 1H, *J* = 12.5, 4.5 Hz), 2.45 (dd, 1H, *J* = 13.5, 6.5 Hz), 2.43 (s, 3H), 2.38 (td, 1H, *J* = 9.5, 2.5 Hz), 2.25 (td, 1H, *J* = 12.5, 3.5 Hz), 1.98 (s, 3H), 1.92-2.02 (m, 1H), 1.72-1.79 (m, 1H), 1.68 (dd, 1H, *J* = 12.5, 2.0 Hz), 1.45-1.53 (m, 1H), 0.78-0.92 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.5, 157.8, 133.4, 130.6, 128.7, 123.6, 107.5, 90.2, 59.2, 46.8, 45.6, 42.7, 42.4, 36.9, 35.1, 23.6, 21.8, 20.3, 20.2; HRMS ($ES^+$) calcd for $C_{19}H_{24}ClN_2O_2$ 347.1526, found 347.1527.

**_N_-((4_S_,4a_R_,7_S_,7a_R_,12b_R_)-11-Chloro-3-methyl-9-nitro-2,3,4,4a,5,6,7,7a-octahydro-1_H_-4,12-methanobenzofuro[3,2-_e_]isoquinolin-7-yl)acetamide 30)**

To a solution of **29** (211 mg, 0.608 mmol) in trifluoroacetic acid (4 mL) at 0 °C was added $NaNO_2$ (80 mg, 1.16 mmol), and the mixture was stirred at 0 °C for 4 h.[39] The reaction mixture was cooled in an ice-water bath, basified with 28% $NH_4OH$, and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried over $MgSO_4$ and the solvent was removed in vacuo to give a crude product that was purified by silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 95:5:1) to give **30** (180 mg, 76% yield) as a syrup. $[\alpha]_D^{20}$ -68.9° (*c* 1.12, $CHCl_3$); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.96 (s, 1H), 5.94 (d, 1H, *J* = 4.0 Hz), 4.93 (d, 1H, *J* = 3.5 Hz), 4.25-4.33 (m, 1H), 3.20 (dd, 1H, *J* = 6.0, 2.5 Hz), 2.96 (d, 1H, *J* = 19.5



Organic & Biomolecular Chemistry Accepted Manuscript

Hz), 2.54 (dd, 1H, *J* = 12.5, 4.0 Hz), 2.42 (dd, 1H, *J* = 12.5, 6.0 Hz), 2.40 (s, 3H), 2.35-2.41 (m, 1H), 2.12 (td, 1H, *J* = 12.5, 4.5 Hz), 2.02 (s, 3H), 1.95-2.04 (m, 1H), 1.76-1.87 (m, 1H), 1.54 (d, 1H, *J* = 12.5 Hz), 1.52-1.60 (m, 1H), 0.75-0.85 (m, 2H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.8, 152.8, 141.7, 135.3, 130.4, 124.6, 123.8, 93.6, 58.9, 46.5, 45.3, 43.3, 42.9, 37.3, 35.2, 23.6, 21.5, 20.7, 20.4. HRMS ($ES^+$) calcd for $C_{19}H_{23}ClN_3O_4$ 392.1377, found 392.1375.

**_N_-((4*S*,4a*R*,7*S*,7a*R*,12b*R*)-9-Amino-3-methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinolin-7-yl)acetamide (31)**

10% (w/w) Pd/C (50 mg) was added to a solution of **30** (85 mg, 0.217 mmol) in 20% aqueous acetic acid (50 ml) and the mixture was stirred under $H_2$ (45 psi) overnight at room temperature. The reaction mixture was filtered through a Celite pad, and concentrated in vacuo to afford a crude product that was purified by silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 90:10:1) to give **31** (52 mg, 73%).

$[\alpha]_D^{20}$ -153.4° (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 6.50-6.56 (m, 2H), 5.80 (d, 1H, *J* = 9.2 Hz), 4.59 (d, 1H, *J* = 4.4 Hz), 4.18-4.26 (m, 1H), 3.48 (s-br, 2H), 3.06 (dd, 1H, *J* = 6.0, 2.8 Hz), 2.94 (d, 1H, *J* = 19.5 Hz), 2.47 (dd, 1H, *J* = 12.5, 4.4 Hz), 2.37 (s, 3H), 2.30-2.40 (m, 1H), 2.21-2.30 (m, 2H), 1.95 (s, 3H), 1.90 (td, 1H, *J* = 12.0, 4.4 Hz), 1.63-1.70 (m, 2H), 1.40-1.50 (m, 1H), 0.95-1.06 (m, 1H), 0.82-0.92 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 169.4, 146.1, 128.8, 127.0, 125.8, 119.4, 116.7, 89.7, 59.7, 46.7, 46.4, 43.2, 42.8, 37.5, 37.0, 23.5, 22.6, 20.3, 19.9; HRMS ($ES^+$) *m/z*: $[M + H]^+$ calcd for $C_{19}H_{26}N_3O_2$ 328.2025, found 328.2012.

**_N_,_N_'-((4*S*,4a*R*,7*S*,7a*R*,12b*R*)-3-Methyl-2,3,4,4a,5,6,7,7a-octahydro-1*H*-4,12-methanobenzofuro[3,2-*e*]isoquinoline-7,9-diyl)diacetamide (32)**



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To a stirred solution of **31** (97 mg, 0.296 mmol) in dry $CH_2Cl_2$ (4 mL) at 0 $^oC$ was added triethylamine (90 mg, 0.888 mmol) and acetic anhydride (60 mg, 0.592 mmol). The resulting solution was warmed to room temperature slowly and stirred for 24 h. The solution was concentrated in vacuo and the residue was purified by silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 95:5:0.5) to give **32** (98 mg, 90% yield) as a pale solid. Crystallization from $CH_2Cl_2$-THF gave colorless crystals. Mp 210-212 $^oC$.; $[\alpha]_D^{20}$ -151.2° (*c* 0.68, MeOH); $^1H$ NMR (500 MHz, $CDCl_3$: $\delta$ 7.30-7.34 (m, 2H), 6.65 (d, 1H, $J$ = 8.0 Hz), 6.47 (d, 1H, $J$ = 9.0 Hz), 4.63 (d, 1H, $J$ = 4.5 Hz), 4.18-4.26 (m, 1H), 3.07-3.131 (m, 1H), 2.97 (d, 1H, $J$ = 18.5 Hz), 2.49 (dd, 1H, $J$ = 12.0, 4.0 Hz), 2.42 (dd, 1H, $J$ = 19.0, 4.8 Hz), 2.38 (s, 3H), 2.29 (t, 1H, $J$ = 9.0 Hz), 2.17-2.25 (m, 1H), 2.17 (s, 3H), 1.88-2.05 (m, 2H), 1.97 (s, 3H), 1.64-1.76 (m, 2H), 1.35-1.45 (m, 1H), 0.96-1.05 (m, 1H), 0.81-0.88 (m, 1H); $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 169.8, 168.5, 150.5, 133.1, 130.0, 123.4, 119.4, 117.7, 90.8, 59.9, 46.5, 46.3, 43.3, 42.7, 37.0, 36.4, 24.1, 23.5, 23.0, 20.5, 20.2; HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{28}N_3O_3$ 370.2131; found: 370.2130. Anal. Found: C, 68.21; H, 7.82; N, 9.07. Calcd for $C_{21}H_{27}N_3O_3$•1.25THF: C, 67.95; H, 8.11; N, 9.14%.

Single-crystal X-ray diffraction data on compound **32**, CCDC deposition number 1002920. Data were collected using CuKα radiation and a Bruker Platinum-135 CCD area detector. The crystal was prepared for data collection by coating with high viscosity microscope oil. The oil-coated crystal was mounted on a micro-mesh mount (Mitergen, Inc.) and transferred to the diffractometer and a data set collected at 150°K. The 0.408 x 0.311 x 0.218 $mm^3$crystal was orthorhombic in space group P $2_12_12_1$, with unit cell dimensions a = 9.1670(2), b = 13.2568(3), and c = 15.2701(4) Å. Data was 94.0% complete to 67.99° θ (~ 0.83 Å) with an average redundancy of 5.47. The final anisotropic full matrix least-squares refinement on $F^2$ with 192 variables converged at R1 = 4.49%, for the observed data and wR2 = 12.36% for all data. The structure was solved by direct methods and refined by full-matrix least squares on F2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI). Corrections were applied for Lorentz, polarization, and

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absorption effects. Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C-H distance set at 0.96 Å. Complete information on data collection and refinement is available in the supplemental material.

**_N_,_N_'-((4_S_,4a_R_,7_S_,7a_R_,12b_R_)-2,3,4,4a,5,6,7,7a-Octahydro-1_H_-4,12-methanobenzofuro[3,2-_e_]isoquinoline-7,9-diyl)diacetamide (33)**

To a solution of **32** (80 mg, 0.216 mmol) in dry $CHCl_3$ (15 mL) was added $KHCO_3$ (163 mg, 1.62 mmol), $K_2CO_3$ (224 mg, 1.62 mmol) and α-chloroethylchloroformate (463 mg, 3.24 mmol) at room temperature. The resulting solution was heated to reflux for 5 h with monitoring by TLC ($CHCl_3$:MeOH:$NH_4OH$, 9:1:0.1). The solution was cooled, filtered, and the solvent removed in vacuo. The residual material was placed under high vacuum for 2 h, followed by the addition of $CH_3CN$-$H_2O$ (4:1, 0.1% TFA, 6 mL), and stirred for 2 h at room temperature. Acetonitrile was removed in vacuo, and the residue was purified by silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 95:4.5:0.5) to give **33** (48 mg, 62% yield) as a pale solid. $[\alpha]_D^{20}$ -133.3° (*c* 1.80, MeOH); $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 7.35 (d, 1H, $J$ = 8.0 Hz), 6.63 (d, 1H, $J$ = 8.0 Hz), 4.55 (d, 1H, $J$ = 4.5 Hz), 4.10-4.15 (m, 1H), 3.42-3.48 (m, 1H), 2.94 (dd, 1H, $J$ = 14.0, 7.0 Hz), 2.70-2.83 (m, 9H), 2.21-2.25 (m, 1H), 2.10 (s, 3H), 1.89 (s, 3H), 1.78-1.90 (m, 2H), 1.60-1.70 (m, 2H), 1.30-1.38 (m, 1H), 1.14-1.20 (m, 1H), 0.92-1.02 (m, 1H), 0.74-0.80 (m, 1H); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{20}H_{26}N_3O_3$ 356.1974, found 356.1964.

**_N_,_N_'-((4_S_,4a_R_,7_S_,7a_R_,12b_R_)-3-(4-(3-(tritylthio)propanamido)butyl)-2,3,4,4a,5,6,7,7a-octahydro-1_H_-4,12-methanobenzofuro[3,2-_e_]isoquinoline-7,9-diyl)diacetamide (3, DiAmHap)**

To a solution of **33** (60 mg, 0.169 mmol) in dry DMF (4 mL) was added $K_2CO_3$ (94 mg, 0.676 mmol), NaI (2 mg) and **36** (98 mg, 0.203 mmol) at room temperature. The resulting solution was warmed to 50 $^oC$ overnight, cooled to room temperature, diluted with 50 mL of ice-water and extracted with $CHCl_3$



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(3x20 mL). The combined organic layers were dried with $MgSO_4$ and filtered before removal of the solvent in vacuo. The residue was purified by silica gel chromatography ($CHCl_3$-MeOH-$NH_4OH$, 90:10:1) to give **3** (82 mg, 64% yield). The solid was lyophilized with *t*-BuOH and a pale white solid was obtained. $[\alpha]_D^{20}$ = -105.1° (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDC1_3$) $\delta$ 8.15 (brs, 1H), 7.38 (d, *J* = 7.6 Hz, 6H), 7.23 (m, 6H), 7.16 (m, 3H), 6.89 (d, *J* = 8.8 Hz, 1 H),6.59 (d, *J* = 8.0 Hz, 1H), 6.04 (brs, 1H), 4.56 (s, 1H), 4.26 (brs, 1H), 3.13 (m, 3H), 2.83 (d, *J* = 18.8 Hz, 1 H), 2.70 (s, 1H), 2.41 (m, 6H), 2.19 (t, *J* = 8.8 Hz, 1H), 2.10 (m, 4H), 2.04 (t, *J* = 6.4 Hz, 2H), 1.94 (s, 3H), 1.82 (t, *J* = 10 Hz, 1H), 1.61 (m, 2H), 1.45 (m, 4H), 1.33 (m, 1H), 0.96 (m, 1H), 0.79 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 171.0, 169.8, 168.7, 150.5, 144.6,132.8, 130.0, 129.5, 127.8, 126.6, 123.5, 119.1, 117.7, 90.4, 66.7, 57.8, 54.4, 46.4, 44.2, 43.0, 39.3, 36.8, 35.8, 35.5, 27.7, 27.3, 24.9, 23.7, 23.3, 22.7, 20.9, 20.5; HRMS ($ES^+$) *m/z*: $[M + H]^+$ calcd for $C_{46}H_{53}N_4O_4S$, 757.3788; found 757.3776. Anal. Found: C, 70.29; H, 7.82; N, 6.60. Calcd for $C_{46}H_{52}N_4O_4S$•1.25$H_2O$•*t*-BuOH: C, 70.35; H, 7.62; N, 6.56%.

**2,2,2-Trifluoroacetaldehyde, 4-bromobutan-1-aminium salt (35)**

To a stirred solution of *tert*-butyl (4-bromobutyl)carbamate (**34**, 252 mg, 1.0 mmol) in anhydrous $CH_2Cl_2$ (2 mL) at room temperature was added trifluoroacetic acid (1 mL) in one portion. The resulting solution was stirred at room temperature for 2 h. The solvent was removed in vacuo to give crude **35** that was used in the next step without further purification. HRMS ($ES^+$) *m/z*: $[M + H]^+$ calcd for $C_4H_{11}N$, 152.0075, found: 152.0071.

***N*-(4-Bromobutyl)-3-(tritylthio)propanamide (36)**

The salt **35** (~ 1.0 mmol) was dissolved in dry $CH_2Cl_2$ (5 mL) and the solution was cooled to 0 °C. To the solution was added 2,5-dioxopyrrolidin-1-yl 3-(tritylthio)propanoate (445 mg, 1.0 mmol), followed by triethylamine (0.42 mL, 3.0 mmol). The mixture was stirred at 0 °C for 2 h and then warmed to



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room temperature overnight. After concentration in vacuo, the residue was purified by silica gel chromatography (hexanes-EtOAc, 70:30) to give **36** (310 mg, 64% yield) as a syrup. $^{1}$H NMR (500 MHz, CDC1$_3$) $\delta$ 7.43 (d, 6H, $J$ = 7.5 Hz), 7.29 (t, 6H, $J$ = 7.5 Hz), 7.22 (d, 3H, $J$ = 7.5 Hz), 5.45 (s-br, 1H), 3.39 (t, 2H, $J$ = 7.0 Hz), 3.21 (q, 2H, $J$ = 6.5 Hz), 2.49 (t, 2H, $J$ = 7.0 Hz), 2.02 (t, 2H, $J$ = 7.5 Hz), 1.82-1.87 (m, 2H), 1.58-1.64 (m, 2H), 1.26 (t, 2H, $J$ = 7.5 Hz); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 171.1, 144.7, 129.6, 128.0, 126.8, 66.9, 38.6, 35.7, 33.4, 30.0, 28.3, 27.9; HRMS (ES$^+$) *m/z*: $[M + H]^+$ calcd for $C_{26}H_{28}NOSBrNa$, 504.0973, found 504.0961.

**Biological Assays**

**Materials**

Bovine serum albumin (BSA), used as blocking reagent for ELISA, morphine sulfate, codeine, trifluoroacetic acid and triisopropylsilane were purchased from Sigma-Aldrich (Saint Louis, MO). 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG), 1,2-dimyristoyl-sn-glycero-3-phosphate (DMPC), monophosphoryl lipid A (PHAD$^{TM}$) (MPLA), and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL). Heroin HCl and 6-acetylmorphine were from Lipomed Inc. (Cambridge, MA). Tetanus toxoid (TT) was purchased from Statens Serum Institut (Copenhagen, Denmark). BSA, used for coupling to haptens, SM-(PEG)$_2$ linker and BCA protein assay kits were purchased from Pierce Protein Research/Thermo Fisher Scientific (Rockford, IL). Immulon$^{TM}$ 2HB flat ELISA plates were purchased from Thermo Scientific (Marietta, OH). Peroxidase-linked sheep anti-mouse IgG (γ□chain specific) was purchased from The Binding Site (San Diego, CA). 2,2'-Azino-di(3-ethylbenzthiazoline-6-sulfonate) peroxidase substrate system was purchased from KPL, Inc. (Gaithersburg, MD).

**Preparation of vaccine formulation and immunization**

6-PrOxyHap (**1**), DiPrOxyHap (**2**), and DaAmHap (**3**) were coupled to BSA as described.[2, 12] Briefly, the trityl protection was removed from the haptens by dissolving in chloroform, treating with



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triisopropylsilane and trifluoroacetic acid for 2 h and the solvent was removed under vacuum overnight. SM-$(PEG)_2$ was incubated with TT for 2 h and dialyzed against PBS, pH 7.4 at 4 °C overnight. The haptens were solubilized in water and added to the TT. The reaction was incubated for 2 h at room temperature with shaking and dialyzed against PBS, pH 7.4 at 4 °C overnight. The protein concentration was determined using BCA. The number of haptens attached per molecule of TT was determined using MALDI-TOF mass spectrometry. Desalted tetanus toxoid conjugates were mixed in a 1:1 ratio with sinapinic acid and spotted on the stainless plate using the sandwich method. Mass spectrum for the conjugates was acquired using AXIMA Mega TOF™ (Shimadzu Scientific Instruments, Columbia, MD). The number of DiAmHap, 6-PrOxyHap, and DiPrOxyHap molecules attached per molecule of TT was 17, 20 and 18, respectively. Liposomes were prepared as described[40] with DMPC:cholesterol:DMPG in a molar ratio of 9:7.5:1. The liposomes were mixed with hapten-TT to give a final dose of 10 µg TT in 0.05 ml. The final phospholipid concentration was 50 mM with 20 µg MPLA per dose in PBS, pH 7.4. Female Balb/c mice from Jackson Laboratories (Bar Harbor, ME) (5-6 weeks of age; 13 per group) were immunized with the liposome-hapten-TT mixture at 0 and 6 weeks in alternate hind thighs. The animals were bled prior to immunization and at weeks 3 and 6 post immunization. Five mice per group were terminally bled at week 9 by cardiac puncture. The remaining 8 mice per group were used for the antinociception assays.

**ELISA and competitive ELISA**

The haptens were coupled to BSA using the same method described above for coupling to TT. The ELISA and competitive ELISA were conducted as described with 0.1 µg hapten-BSA per well.[2, 12] Briefly, the hapten-BSA was added to the plates and incubated at 4 °C overnight. The plates were blocked with 20 mM Tris-0.15M sodium chloride-1% BSA (blocker), pH 7.4 for 2 h. The blocker was removed and sera, serially diluted in blocker, were added to the plates. Following incubation for 2 h,

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the plates were washed with 20 mM Tris-0.15M sodium chloride-0.05% Tween 20®. Peroxidase linked anti-mouse IgG diluted in blocker was added and the plates were incubated for 1 h at room temperature. The plates were washed and ABTS was added. After incubation for 1 h, the absorbance was read at 405 nm. For the competitive ELISA, sera were diluted to give an absorbance of approximately 1.5. Heroin HCl, codeine, and morphine sulfate were dissolved in blocker, diluted in 10-fold concentrations (Fig. 6) and mixed with diluted sera. 6-Acetylmophine was dissolved in DMSO at a 10-fold higher concentration prior to dilution in blocker and mixing with diluted sera. The sera were incubated for 1 h at room temperature with the inhibitors and then were added to the ELISA plates, which were processed as described above.

**Nociception assay**

The hot plate test was used to measure the heroin-induced antinociception of the immunized animals.[12, 41] The hot plate (Harvard Apparatus, Holliston, MA) was set at 58 °C and the time was measured until the animal lifted one of its hind paws or jumped. A cut-off of 30 sec was set to prevent burns. At week 9, 8 of the 13 mice were selected and the base line response was measured. Heroin HCl (0.75 mg/kg) in saline (300 μg/ml) was injected by the subcutaneous route between the front shoulders. After 20 min, the hot plate response was measured again.

**Data analysis**

Antibody endpoint titers were defined as the dilution at which the absorbance was twice background. Background absorbance was 0.05 – 0.1. Values from the individual animals were averaged for the group endpoint titer shown in Fig. 1. GraphPad Prism was used for statistical analysis. $IC_{50}$ values were calculated using nonlinear regression, one-sit-fit log $IC_{50}$ model as described (Matyas et al.,

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2014). The nociception data were convert to the percent maximum potential effect (% MPE) using the formula:

$$\% \text{ MPE} = 100 \times \frac{(\text{injection latency time} - \text{baseline latency time})}{(\text{cut-off-baseline latency time})}$$

The data for the immunized mice were compared to the naïve unimmunized control mice using the T-test (unpaired, one tail).

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Army, Department of Defense, or NIH, or the U.S. Government.

**Supporting Information Available:** Spectroscopic data, and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org. Atomic coordinates for **8b**, **13a**, **27, and 32** have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers CCDC 975660, 954929, 955757, and 1002920, respectively). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk.

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