# Polymers for the Sustained and Localized Release of Anti-inflammatory and Anti-cancer Drugs

by

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# Abstract of the Thesis

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The practical utility of chemically incorporating drugs into polymers for the localized and sustained delivery of such drugs has been well established. Sustained release provides therapeutic concentrations of drug over a long time period, obviating the need for frequent dosing, leading to better patient compliance and mitigating the likelihood of overdosing. Localized delivery mitigates the severity of side effects associated with systemic administration and increases efficacy by increasing the effective dosage at the site of the pathology. In this thesis, these principles have been investigated for the delivery of anti-inflammatory and anti-cancer drugs.

Amfenac, a non-steroidal anti-inflammatory drug (NSAID), in the form of its prodrug nepafenac, has been proven to be effective for the treatment of anterior uveitis (i.e. ocular inflammation) via topical administration of an aqueous suspension, but posterior uveitis is difficult to treat in this manner as drug penetration to the rear of the eye is inefficient and

clearance is very rapid, limiting the practicality of administration by injection. The objective was to chemically incorporate amfenac into a biodegradable polymer that can be fabricated into microspheres suitable for injection into the rear of the eye for the sustained release of amfenac to treat posterior uveitis. This polymer consists of a poly(amide-imine) derived from an analog of nepafenac. Herein is described the polymer synthesis and the preparation and degradation of PolyAspirin™ microspheres incorporating sodium amfenac.

Nutlin is a member of an anti-cancer drug family developed by Hoffmann-LaRoche, Inc. The objective of this project was to attach a nutlin analog to a biodegradable polymer backbone and fabricate the resulting polymer into wafers that could be surgically implanted to treat brain cancer. Described herein are efforts to incorporate a nutlin analog into a polymer backbone based on pyromellitic acid.

# Preface

"Man's work must ever end in failure, unless it bears the stamp of mind. The head must plan with care and thought, before the hand can execute." Schiller

# Acknowledgements

Thanks to:

Dr. Uhrich, my committee members and the members of the Uhrich group.

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# 1. Introduction

Polymers are ubiquitous in today's world, finding use in everything from food containers to teeth fillings, and they have also found use as drug-delivery vehicles.[1-3] Polymers as drug-delivery vehicles offer many advantages: delivery can be sustained over a long time period, obviating the need for frequent dosing and increasing patient compliance. This aspect is particularly relevant in the case of drugs that need to be administered by injection, which can be painful. Polymers can also be fabricated into many forms suitable for administration by various means (e.g. surgical implantation, injection) for localized delivery (**Figure 1**), minimizing the side-effects associated with systemic administration and increasing efficacy at the intended site of administration.[1] Polymers can also be tailored to release the drug in response to a particular biological environment.[2]

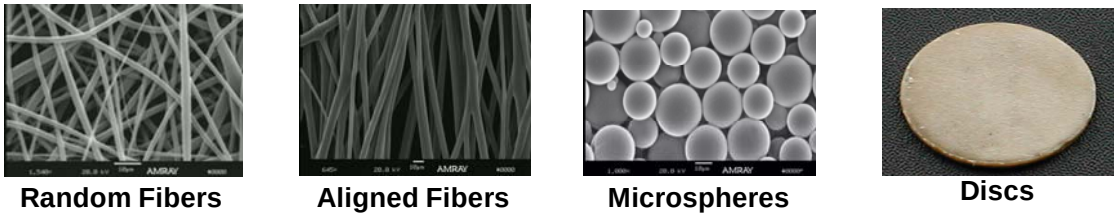



**Figure 1**: Some fabricated forms of PolyAspirin.[4-8]

Three methods of incorporating drugs into polymers are described: physical incorporation (**Figure 3A**), pendant attachment (**Figure 3B**) and chemical incorporation (**Figure 3C**). "Physical incorporation" is the most generally applicable as the drug is simply physically mixed with the polymer and diffused from the polymer matrix over

time or, in the case of a biodegradable polymer, is released as the polymer degrades. Drug loadings in excess of 50% have been achieved with this method.[9]

The "pendant attachment" method involves attaching a suitable drug molecule to a polymer backbone via a covalent bond which will be cleaved under physiological conditions. This method requires that the drug molecule have a suitable functional group by which to effect attachment.

The third method of incorporation is "chemical incorporation", in which the drug is chemically incorporated into the polymer backbone via two degradable covalent bonds. Usually, a divalent linker molecule is used between drug molecules enabling some control over the properties of the polymer. Very high drug loadings, theoretically as high as 100% if no linker molecule is employed, can be achieved by this method, but it requires that the drug have two functionalities for covalent linkage. All three methods of incorporation are illustrated in **Figure 2**:

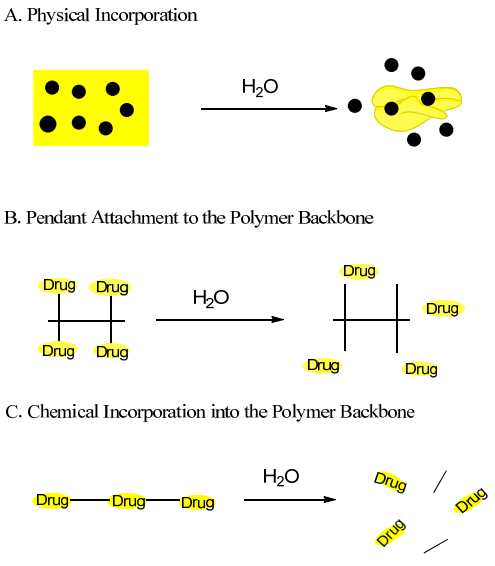


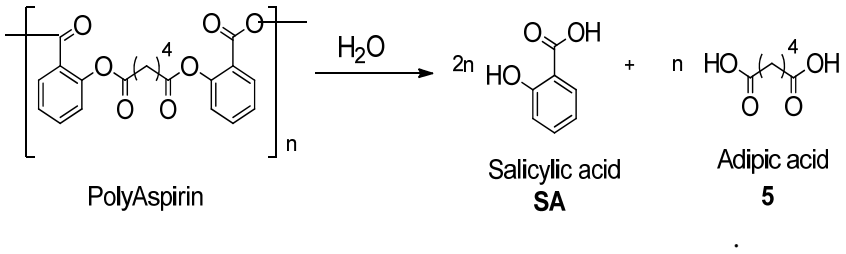

**Figure 2**: A. Drug is released upon polymer degradation. B. Drug is attached to a polymer backbone and released upon hydrolytic cleavage. C. Drug is incorporated into a polymer backbone via two labile covalent bonds and released upon hydrolytic cleavage.

Polymers used for *in vivo* drug delivery should be non-toxic and biodegrade into non-toxic components so that no residue remains and a high percentage of the incorporated drug is released. These requirements place strict limitations on the types of polymers suitable for drug delivery and currently much research is underway to discover such polymers.[10]

A recent example of such research is work performed by Murthy *et al*.[11] utilizing polyketals for the sustained release of imatinib, an anti-inflammatory agent. In this work, a series of random copolymers were synthesized from 1,4-cyclohexanedimethanol, dimethoxypropane and aliphatic terminal diols with carbon numbers of four to eight, in various ratios which produced polymers of different degrees of hydrophobicity. The polymer degradation rate paralleled the degree of hydrophobicity, thus demonstrating their tunability. Polyketals are noteworthy in that they are stable at the intercellular pH of 7.4 and do not hydrolyze until incorporation into acidic endosomes.[11]

Another class of polymers which have proven particularly advantageous for drug delivery is polyanhydrides.[12] Polyanhydrides have the property of undergoing surface erosion as opposed to bulk erosion, leading to more consistent drug release.[13] Anhydrides are rapidly hydrolyzed relative to permeation of the polymer matrix by the degradation medium. Thus, the bulk integrity of the matrix is maintained while erosion occurs primarily at the surface.[14]"Erosion" as used here is defined as the physical breakdown of the polymer matrix, whereas "degradation" is defined as the chemical breakdown of the polymer.

An example of a polyanhydride used for sustained release is PolyAspirin$^{TM}$, first prepared by Uhrich *et al*. for the sustained release of salicylic acid (SA) (**Scheme 1**).[4] In this polymer, salicylic acid, a non-steroidal anti-inflammatory drug, is chemically incorporated into a polyanhydride and slowly released upon hydrolytic degradation of the polymer. The degradation rate can be controlled by varying the nature of the diacid used to link the salicylic acid molecules, which in this example is adipic acid (**5**).[7]




**Scheme 1**: Chemical structure of PolyAspirin and its degradation products.

The technology described above is applicable to a variety of drugs containing suitable functional groups. Specifically, herein will be described efforts to incorporate the non-steroidal anti-inflammatory drug amfenac into a polymer for the treatment of posterior uveitis and an anti-cancer drug of the nutlin family into a polymer for the treatment of brain cancer.

# 2. Polymers for the Sustained Release of Amfenac for Treating Posterior Uveitis

## 2.1 Introduction

Ocular inflammation (uveitis) is a leading cause of blindness, affecting over two million people worldwide and 109,000 in the U.S. alone, with 43,000 new cases diagnosed annually.[16, 17] Uveitis can occur in the anterior (front), posterior (rear), in between (intermediate) or throughout the eye (pan-uveitis) and has various causes. The most advantageous treatment for anterior uveitis is the topical administration (eyedrops) of amfenac (**1**), in the form of its prodrug nepafenac (**2**, **Figure 3**).[18] However, topical administration of nepafenac is not an effective treatment for posterior uveitis as drug penetration to the posterior of the eye is inefficient[17] and administration by injection is impractical as clearance of the drug is too rapid[17,19], necessitating daily injections, a daunting prospect for even the bravest of patients.

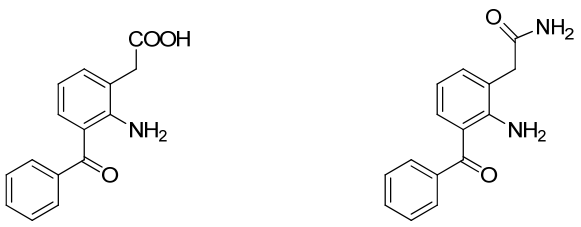



**Figure 3**: Chemical structures of amfenac (**1**) and nepafenac (**2**).

The current standard of treatment for posterior uveitis is the administration of corticosteroids topically or intraocularly by injection.[20] However, they have a high incidence of serious side-effects such as glaucoma, cataracts, secondary infections due to

immunosuppression and bone loss.[17, 20] While nepafenac is effective for treating anterior uveitis, and likely would also prove effective for the treatment of posterior uveitis, no practical way of delivering the drug to the posterior of the eye is yet available. If nepafenac can be incorporated into a slowly degrading polymer and fabricated into injectable microspheres, it should prove effective for the treatment of posterior uveitis as well.

## 2.2 Attempted Synthesis of Amfenac Poly(adipamide-anhydride)

Early efforts were directed at taking advantage of the structural similarity between amfenac and salicylic acid to synthesize a polyanhydride incorporating amfenac. It was envisioned that a diacid (e.g. adipic acid) could be used to chemically link amfenac molecules and the resulting diacid would then be polymerized to a polyanhydride as had been done with salicylic acid (**Figure 4**).[4]

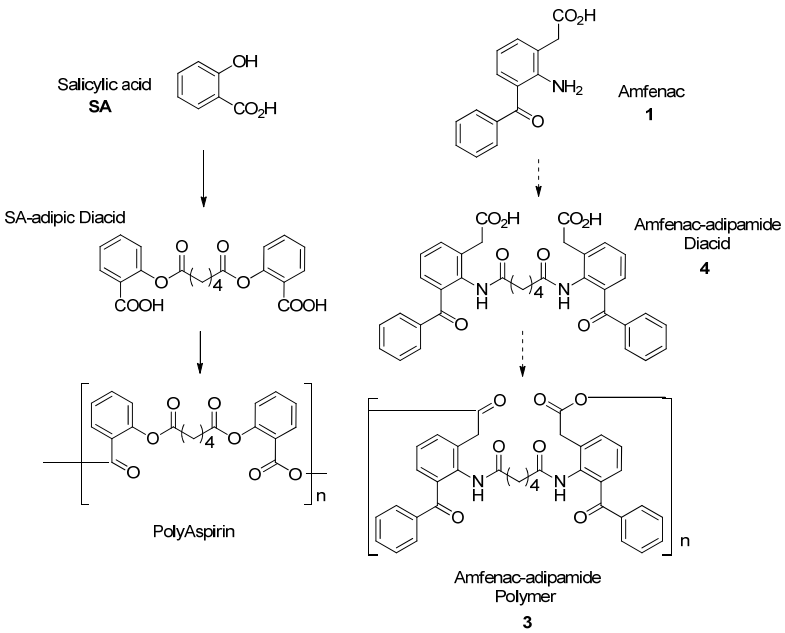
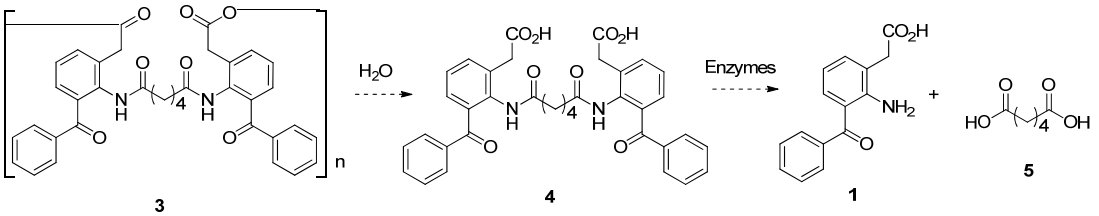



**Figure 4**: Analogous strategy to PolyAspirin synthesis.

It was envisioned that the amfenac-adipamide polyanhydride (**3**, **Scheme 2**) would hydrolytically degrade *in vivo* to release the amfenac diacid (**4**), which in turn will degrade into amfenac (**1**) and adipic acid (**5**) in a manner analogous to PolyAspirin.

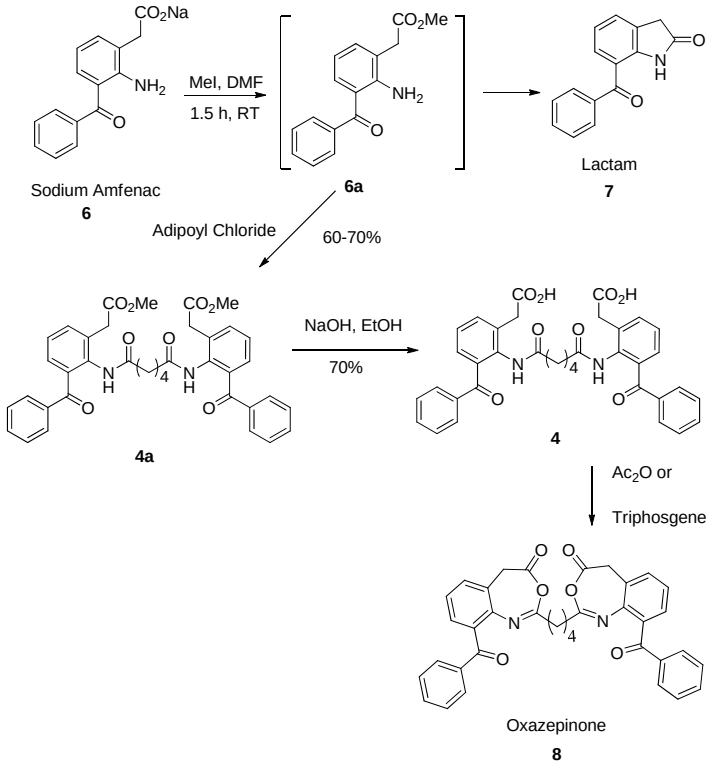



**Scheme 2**: Proposed hydrolytic degradation of the proposed amfenac-adipamide polyanhydride (**3**).

A significant difference between the proposed polymer and PolyAspirin is that the ester linkages in PolyAspirin are known to undergo hydrolytic cleavage *in vivo* without the need for enzyme catalysis.[21] Amides are considerably more hydrolytically stable than esters and require enzyme catalysis for hydrolysis under physiological conditions.[21] Thus, the degradation of the intermediate diacid **4** would require catalysis by amidases.

The attempted synthesis of **3** is depicted in **Scheme 3** below, beginning with sodium amfenac (**6**), the commercially available form of amfenac. Sodium amfenac (**6**) was alkylated with methyl iodide in dimethylformamide (DMF) to give the methyl ester of amfenac (**6a**).[22] Compound **6a** may be isolated, but is prone to cyclize to the lactam (**7**)[23], which is the major byproduct of the reaction as determined by thin-layer chromatography (10% MeOH/$CH_2Cl_2$) and NMR. **6a** was then acylated with adipoyl chloride *in situ* to give the dimethyl ester of the bisamide (**4a)**. Compound **4a** was then hydrolyzed with aqueous NaOH in ethanol to give the diacid **4** after neutralization with Amberlyst 15 resin, as **4** proved to be surprisingly water-soluble and could not be extracted. Unfortunately, attempted polymerization of **4** utilizing melt-condensation with

acetic anhydride or solution polymerization with triphosgene yielded the oxazepinone (**8**) as the sole product as determined by NMR and thin-layer chromatography (5% $MeOH/CH_2Cl_2$).




**Scheme 3**: Attempted synthesis of amfenac-adipamide polyanhydride and unexpected side reactions.

Concurrently, we determined that **4** did not enzymatically degrade when incubated at pHs 7.4 and 4.0 with cathepsins B and D, two amidases known to be present in ocular tissues,[24] and an *ex vivo* analysis performed by Wolfe Laboratories utilizing rabbit retinal/choroidal homogenate was also negative. Finally, a search of the literature revealed that the acetamide of amfenac, which is structurally similar to **4**, had been synthesized and tested i*n vivo* by previous researchers and was found to be inactive.[23] Thus, it appeared that our initial target polymer was not suitable for the desired purpose.

## 2.2a Experimental

**Preparation of methyl amfenac bis-adipamide (4a):**

Sodium amfenac (**6**) (6.10 g, 22.0 mmol), previously dried by azeotroping with toluene, was dissolved in 45 mL of anhydrous DMF. Methyl iodide (2.70 mL, 44.0 mmol, 2.0 eq) was added and the reaction was stirred for 1 h at room temperature under nitrogen. The reaction was then cooled to 0 $^{o}$C and adipoyl chloride (1.60 mL, 11.0 mmol, 0.5 eq) was added dropwise over ten minutes. After the addition, the reaction was warmed to room temperature and stirred for 1 h. The solvent was then removed *in vacuo* and the residue partitioned between 100 mL of ethyl acetate and 50 mL of 1% aqueous sodium sulfite. The phases were separated and the organic phase washed with 4 x 50 mL of water, 25 mL of saturated brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude **4a** thus obtained was triturated with 50 mL of ethyl ether overnight, filtered, washed with ethyl ether and dried *in vacuo*. The product was a pink solid weighing 4.30 g (60.3%), which was used without further purification. $^{1}$H NMR (300 MHz, $CDCl_3$): δ 8.67 (s, 2H), 7.80 (d, 4H), 7.55 (t, 2H), 7.50-7.30 (m, 6H), 7.25 (t, 4H), 3.70 (s, 6H), 3.65 (s, 4H), 2.18 (t, 4H), 1.52 (t, 4H). MS (m/z, M+H): Calcd. For $C_{38}H_{37}N_2O_8$: 649.25: Found: 649.2. IR (KBr, $cm^{-1}$): 3261 (NH, amide), 2947 ($CH_2$, alkane), 1736 (C=O, ester), 1675 (C=O, amide), 1645 (C=O, ketone). M.p.: 159-165 $^{o}$C.

**Preparation of amfenac bis-adipamide diacid (4):**

Compound **4a** (4.30 g, 6.63 mmol) was suspended in 40 mL of 95% EtOH. NaOH (2.10 g, 26.5 mmol) was added and the reaction stirred at room temperature for 4 h. Amberlyst 15 resin (6.63 g) was added and the reaction stirred for ½ h. The reaction

was then filtered and the resin washed with 2 x 25 mL of hot 95% EtOH. The solvent was removed *in vacuo* and the residue triturated with 30 mL of acetone overnight. The residue was then filtered, washed with acetone and dried *in vacuo* over $P_2O_5$. The product so obtained (**4**) was a white solid weighing 2.90 g (70.6%). $^1$H NMR (300 MHz, $CDCl_3$): δ 9.59 (s, 2H), 7.60 (m, 6H), 7.50 (m, 6H), 7.30 (m, 4H), 3.62 (s, 4H), 1.75 (m, 4H), 1.10 (m, 4H). MS (m/z, M-H): Calcd. for $C_{36}H_{31}N_2O_8$: 619.21 Found: 619.2. IR (KBr, $cm^{-1}$): 3259 (NH, amide), 3100-2850 (OH, carboxylic acid), 1725 (C=O, carboxylic acid), 1680 (C=O, amide), 1650 (C=O, ketone). M.p.: 209-211 $^o$C.

**Preparation of the oxazepinone (8):**

Compound **4** (100 mg, 0.161 mmol) was suspended in 2 mL of dry methylene chloride (DCM). Triethylamine (0.100 mL, 0.717 mmol) was added and the solution was cooled to 0 $^o$C under nitrogen. Triphosgene (53.0 mg, 1.10 eq) in 1 mL of DCM was then added over five minutes. The reaction was stirred at 0 $^o$C for ten minutes and warmed to room temperature. After stirring at room temperature for 1 h, the reaction was diluted with 10 mL of DCM, washed with 5 mL of water, dried over $MgSO_4$, filtered and the solvent evaporated *in vacuo*. The product was a pink solid. $^1$H NMR (300 MHz, $CDCl_3$): d 7.85-7.65 (m, 4H), 7.60-7.10 (m, 12H), 3.78 (s, 4H), 2.80 (m, 4H), 1.40 (m, 4H). MS (m/z, M-H): Calcd. for $C_{36}H_{27}N_2O_6$: 583.18 Found: 583.20. IR (KBr, $cm^{-1}$): 2947 (C-H, alkane), 1759 (C=O, lactone), 1710 (C=N, iminoester), 1666 (C=O, aryl ketone).

***Ex vivo* degradation study of compound 4: *Performed by Wolfe Laboratories, Inc.***

***Retina extraction procedure:*** *Compounds were extracted from retina samples by protein precipitation using acetonitrile fortified with 20 ng/mL of internal standard (IS). Retina*

*homogenate was prepared by addition of 2 mM ammonium acetate, 5% methanol (mobile phase A) to retinas to achieve a final retina content of 5% (w/v), followed by homogenization to achieve a visually homogeneous suspension. Four volumes of acetonitrile containing IS were added to one volume of retina homogenate containing one-tenth volume of dimethylsulfoxide (400 μL to 90 μL + 10 μL). The solutions were gently mixed for approximately one minute, followed by centrifugation (3220 rcf, ambient temperature) for ten minutes. A volume (approx. 400 μL) of the resulting supernatant was removed and stored at ambient temperature prior to analysis. Stock solutions of the compounds in dimethylsulfoxide were spiked into blank retina homogenate (10 μL to 90 μL) to obtain a final range of standards from 0.1 to 1000 ng/mL.*

***Degradation study of 4 in rabbit retina/choroid homogenate:*** *Compound* ***4*** *and nepafenac (****2****) were spiked into retina homogenate (5% w/v in 2 mM ammonium acetate, 5% methanol) at a target concentration of 140 μM and incubated at 37 $^{o}$C with mild agitation. Samples were removed at 0, 1, 2, 4, and 24 h, diluted, extracted and analyzed via high pressure liquid chromatography on a Shimadzu LC-20 AD – Applied Biosystems 4000 QTRAP with Analyst Software Version 1.4.2 using a ACE 3 C18 2.1 x 50 mm column. The eluent employed was a gradient of 95:5 water/methanol to 95:5 methanol/water at a flow rate of 0.5 mL/minute.*

## 2.3 Attempted Synthesis of Amfenac-Salicyl Poly(ester-urethane)

Given that **4** was not bioactive, another target polymer was required which could be expected to degrade into amfenac. Polymer (**9**), derived from amfenac and salicylic acid, would serve this purpose, the structure of which is depicted in **Figure 5**. This polymer could conceivably be synthesized from the methyl ester urethane (**10**).

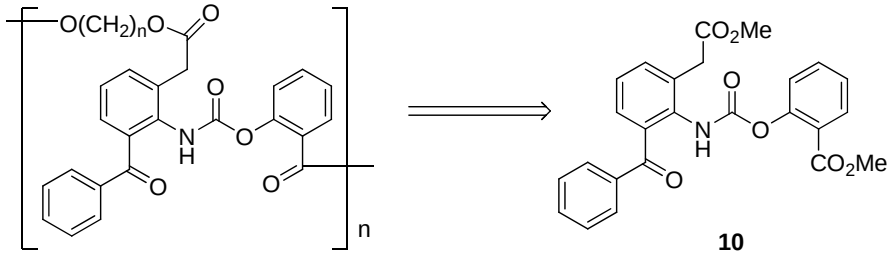
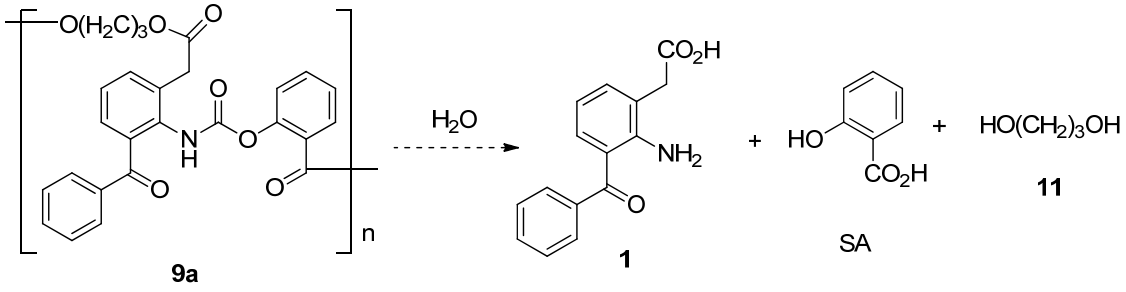



**Figure 5**: Chemical structure of the proposed amfenac-salicyl poly(ester-urethane).

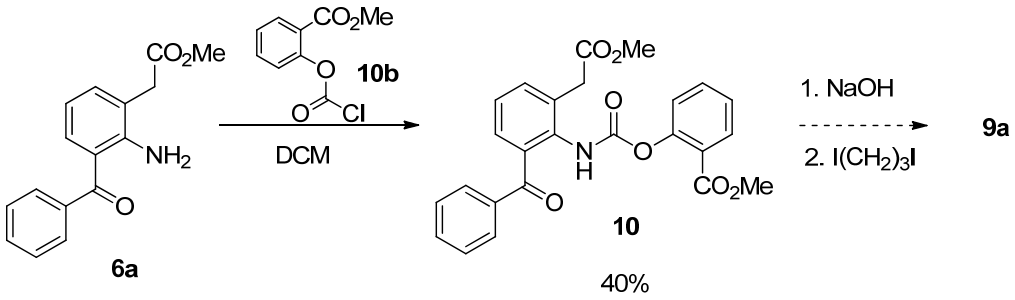
Due to the propensity of amfenac to cyclize to the oxazepinone (**8**), an alternative to a polyanhydride was necessary. Thus, the anhydride linkage would be replaced by an ester, which is also known to be biodegradable.[24] The proposed degradation for the prototypical propyl analog (**9a**, n = 3) is depicted in **Scheme 4**.




**Scheme 4**: Proposed hydrolytic degradation of **9a** into amfenac, salicylic acid and propanediol.

Like esters, aryl urethanes are readily hydrolyzed *in vivo*.[21] Thus, it was envisioned that polymer **9a** would hydrolyze into amfenac, SA and propanediol (**11**). Alkanediols are known to be biocompatible, a notable exception being butanediol.[11]

The proposed synthesis of **9a** is depicted in **Scheme 5**. Alkylation of **6** with methyl iodide, followed by acylation with methyl salicylchloroformate[25] (**10b**), gave **10** in 40% yield from **6**. However, **10** proved to be too hydrolytically unstable, hydrolyzing readily back to methyl amfenac upon chromatography on silica gel or even brief contact with water. Thus, the salicyl urethane (**10**) was deemed too unstable for our purpose.




**Scheme 5**: Attempted synthesis of amfenac-salicyl poly(propyl-urethane) (**9a)**.

## 2.3a Experimental

**Preparation of methyl salicylchloroformate (10b):**

This is a modification of a literature procedure:[25] Methyl salicylate (10.0 g, 65.7 mmol) and triphosgene (19.5 g, 65.7 mmol) were dissolved in 100 mL of dry toluene under argon. The solution was cooled to 0 $^{o}$C and pyridine (5.20 g, 65.7 mmol) was added dropwise with stirring, the temperature being maintained below 15 $^{o}$C. After the addition of the pyridine, the reaction was warmed to room temperature and stirred until the pale yellow precipitate turned white. The reaction was then heated to 75 $^{o}$C and

stirred for 6 h. It was then cooled to room temperature, diluted with 100 mL of hexane and stirred for twenty minutes. The reaction was then filtered and the hexane evaporated *in vacuo.* The product was a colorless liquid weighing 13.3 g (94.3%). $^{1}$H NMR (300 MHz, $CDCl_3$): δ 8.17 (d, 1H), 7.61 (t, 1H), 7.40 (t, 1H), 7.22 (d, 1H), 3.95 (s, 3H). IR (NaCl, $cm^{-1}$): 2954 (C-H, aromatic), 1791 (C=O, chloroformate), 1727 (C=O, ester). MS was inconclusive.

**Preparation of methyl amfenac (6a):**

Sodium amfenac (**6**) (10.0 g, 36.1 mmol), previously dried by azeotroping with toluene, was dissolved in 200 mL of dry DMF under nitrogen. Methyl iodide (3.37 mL, 1.50 eq) was then added and the reaction stirred at room temperature for 1 h. The solvent was then evaporated *in vacuo* at 30 $^{o}$C and the residue partitioned between 50 mL of ethyl acetate and 50 mL of 1% aqueous $Na_2SO_3$. The phases were separated and the organic phase was washed with 4 x 50 mL of water, 25 mL of brine, dried over $MgSO_4$, filtered and the solvent removed *in vacuo*. The product was a yellow crystalline solid weighing 8.65 g (89.0%) which was used without further purification. $^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, 2H), 7.51 (t, 1H), 7.45 (m, 3H), 7.25 (d, 1H), 6.60 (t, 1H), 6.55 (b, 2H), 3.75 (s, 3H), 3.62 (s, 2H). IR (KBr, $cm^{-1}$): 3428 (N-H, amine), 3314 (N-H, amine), 1724 (C=O, ester), 1609 (C=O, ketone), 1552 (N-H, amine).

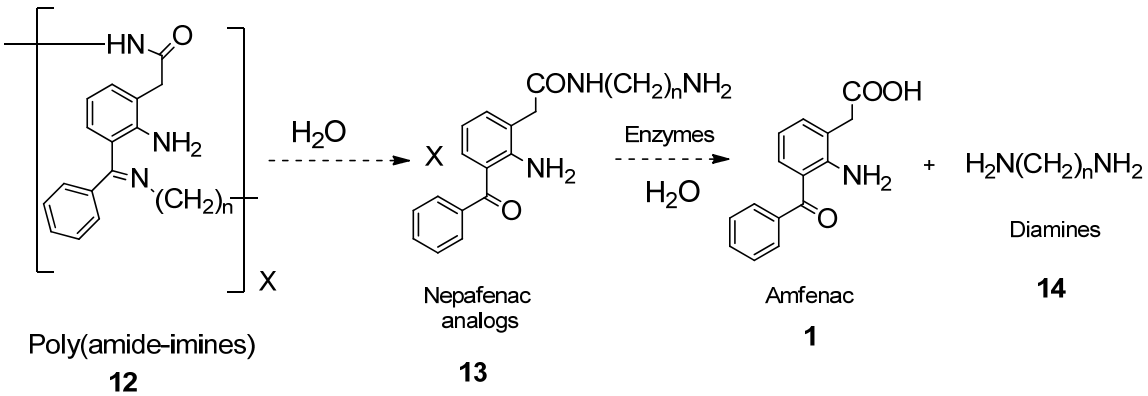
**Preparation of methyl amfenac – salicyl urethane (10):**

Methyl amfenac (**6a**) (100 mg, 0.037 mmol) was dissolved in 2 mL of dry DCM and cooled to 0 $^{o}$C under nitrogen. Methyl salicylchloroformate (**10b**) (80 mg, 0.37 mmol) was added and the reaction stirred at 0 $^{o}$C for 2 h. The solvent was then removed *in vacuo* and the residue chromatographed on silica gel eluting with 5, 10, 15% ethyl

acetate/petroleum ether. The product was a white foam weighing 30 mg (40%). $^1$H NMR (300 MHz, $CDCl_3$, mixture of rotamers): δ 8.61 (b, ½ H), 7.95 (d, 1H), 7.85 (d, 2H), 7.60 (t, 1H), 7.55-7.40 (m, 5H), 7.30 (t, 1H), 7.25 (t, 1H), 7.05 (b, ½ H), 3.92 (b, 2H), 3.75 (s, 3H), 3.68 (s, 3H). MS (m/z, M+Na): Calcd. for $C_{25}H_{21}NO_7Na$, 470.13 Found: 470.1.

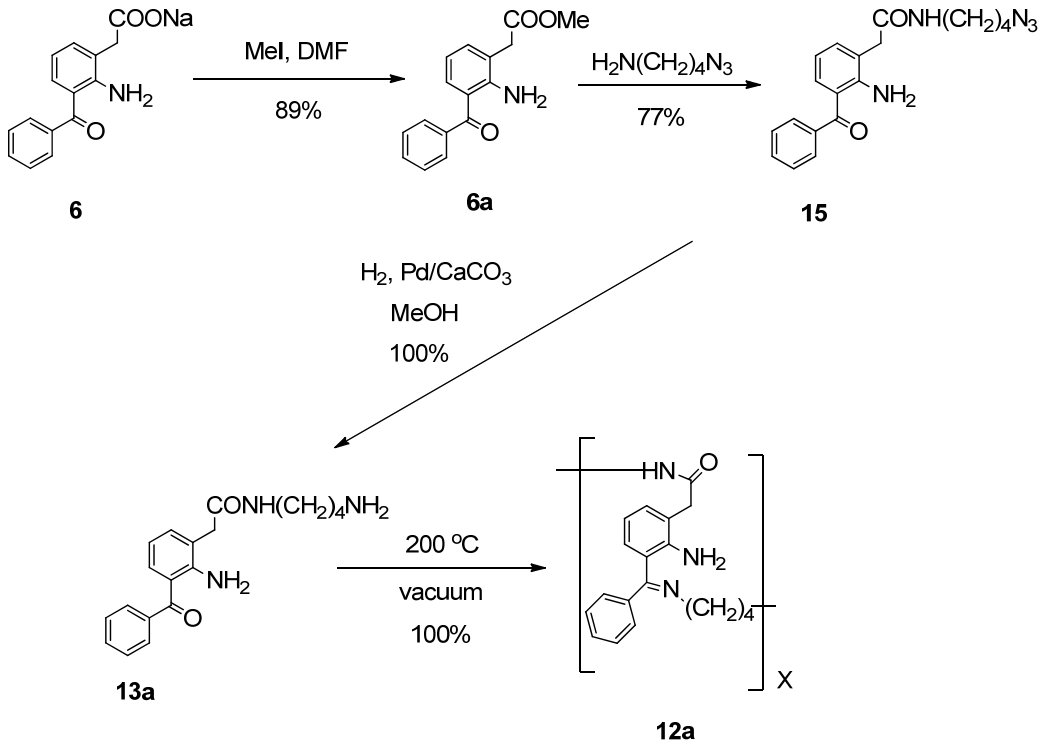
## 2.4 Synthesis of Amfenac Poly(amide-imines)

At this point, alternate strategies to functionalizing the amine were evaluated. Not substituting the amine, however, precluded a polyester, as amfenac esters are unstable towards cyclization to the lactam (**7**). Thus, analogs of nepafenac were conceived, as the amide functionality is perfectly stable to cyclization at ambient temperature and yet degrades enzymatically *in vivo* to amfenac.[24] Specifically, an alkyl-substituted amide with an amine group on the terminal carbon could condense with the carbonyl to form an imine, a functional group which is known to be hydrolytically labile.[21] The structure of the proposed poly(amide-imines) (**12**) is depicted in **Scheme 6,** along with the proposed degradation to amfenac and an alkyl diamine, compounds known to be biocompatible.[26]




**Scheme 6**: Chemical structure of the proposed poly(amide-imines) and their proposed degradation to amfenac and a diamine.

The prototypical target chosen was the butyl analog (**12a**, n = 4). This analog was chosen to mitigate the possibility of the amine condensing with the amide intramolecularly to form a diazepine instead of condensing intermolecularly with the ketone. The ultimately successful synthesis of **12a** is depicted in **Scheme 7**.




**Scheme 7**: Synthesis of amfenac poly(butylamide-imine) (**12a**).

Compound **6** was esterified as before with methyl iodide, giving the methyl ester (**6a**), which was isolated as an unstable yellow crystalline solid in 89% yield. This compound was then reacted with 1,4-azidoaminobutane[27] neat to give the amide-azide (**15**) as a yellow crystalline solid in 77% yield from **6** after purification.[28]

Initially, it was envisioned that the amide-amines could be synthesized directly from alkyl diamines and **6a**. This approach was attempted using ethylenediamine, but the

amide-amine proved to be unstable to chromatography and could not be purified prior to polymerization. It was surmised that the silica gel was catalyzing the amine-ketone condensation; it was subsequently found that **13a** could in fact be chromatographed using 1% aqueous ammonia in methanol/methylene chloride.

Another reason to proceed via the azide (**15**) was to provide an alternative method of polymerization via the phosphazene (derived from the azide and a trialkyl or aryl phosphine) in the event that melt-condensation of the amine proved problematic.

Hydrogenation of the azide (**15**) proceeded in quantitative yield to give **13a** as a yellow crystalline solid with a $T_d$ of 302 $^oC$. Compound **13a** was then heated *in vacuo* at 200 $^oC$ for three hours to give **12a** as an amber glass in quantitative yield. Size-exclusion chromatography indicated this product to be an oligomer with an $M_w$ of 2.5 kDa and $M_n$ of 1.0 kDa. Prolonged reaction time or temperature did not increase the molecular weight.

It remains to be determined if the butylamide-amine (**13a**), or indeed the polymer itself (**12a**), degrades into amfenac under physiological conditions. Qualitatively, **13a** degrades to the lactam at pH 4.0 in the presence of cathepsins B and D at 37 $^oC$, as the lactam was detected after six days using thin-layer chromatography. However, this does not prove that amfenac is an intermediate in that degradation.

The $^1H$ NMRs of the butylamide-amine (**13a**) and the poly(butylamide-imine) (**12a**) are depicted in **Figures 6** and **7**, respectively. Note that the c and d $CH_2$s in **13a** appear to correlate with the broad peak in **12a** at $\delta$ 1.2 - 1.8 and the b, e and g protons in

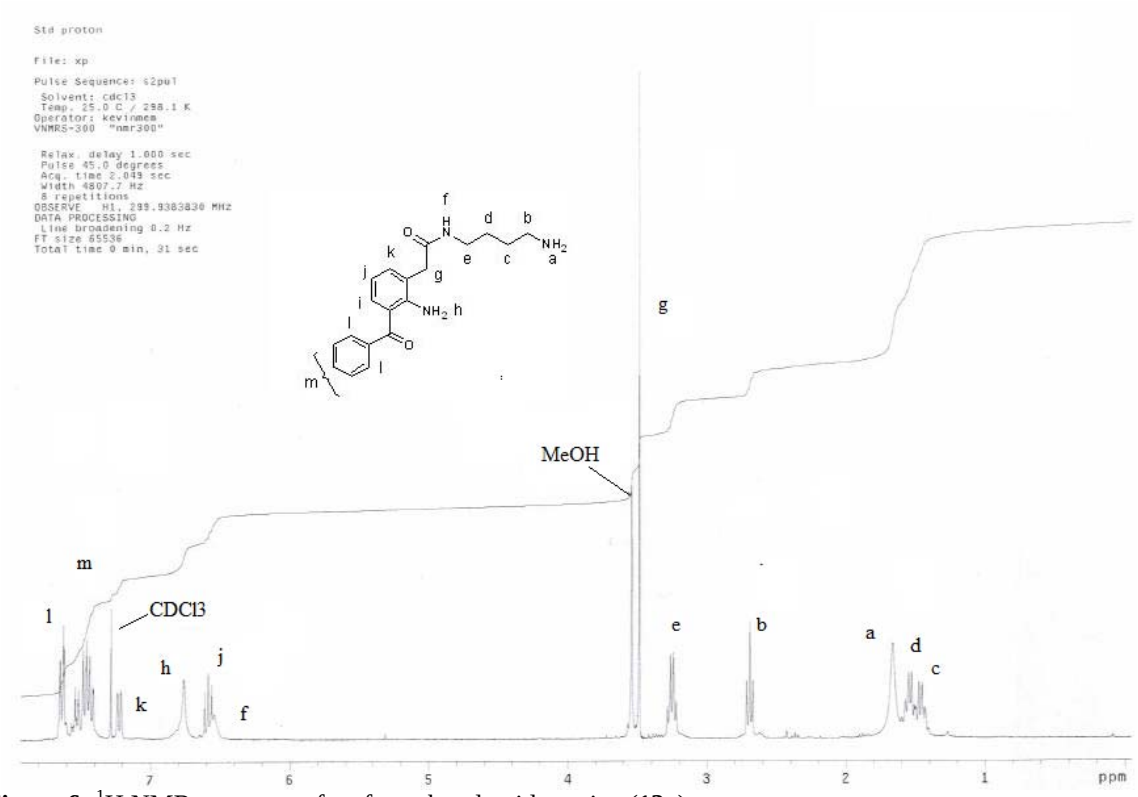



**Figure 6:** $^{1}$H NMR spectrum of amfenac butylamide-amine (**13a**).

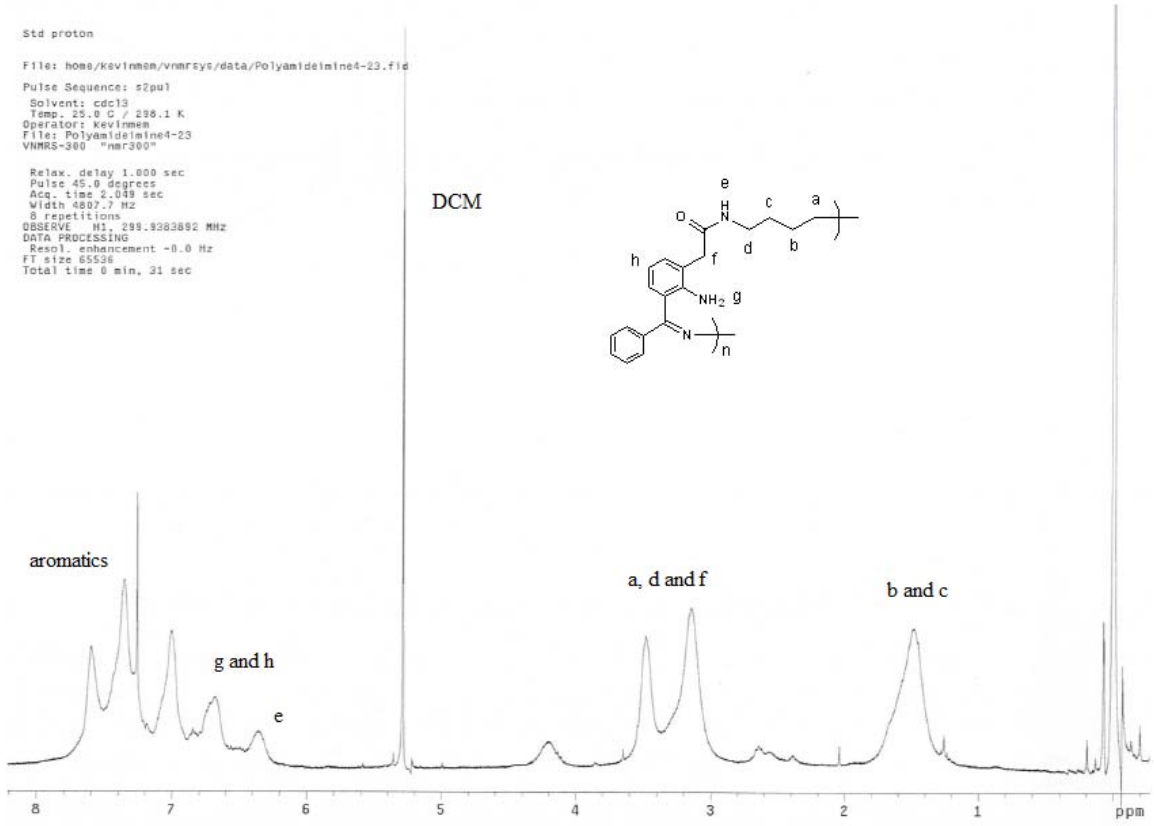



**Figure 7**: $^{1}$H NMR spectrum of the amfenac poly(butylamide-imine) (**12a**).

the monomer correlate with the peak at δ 3.0 – 3.6. The remaining aromatics (j, k, l, i and m), the amine protons (h), and the amide proton (f), correlate with the broad absorbance at δ 6.2 - 7.65. The integration is also in accord with this analysis.

## 2.4a Experimental

**Preparation of amfenac butylamide-azide (15):**

Methyl amfenac (**6a**) (4.00 g, 14.9 mmol) and 1,4-azidoaminobutane (11.0 g, 77.9 mmol) were mixed and stirred overnight at room temperature. The reaction mixture was then triturated with 3 x 40 mL of petroleum ether for 15 minutes. The residual solvent was removed *in vacuo* to give the crude product as a dark oil. The combined supernatants were evaporated *in vacuo* to give 5.5 g of recovered azidoaminobutane which was reacted with another 3.00 g of methyl amfenac and the process repeated to give another batch of crude product and 2.7 g of azidoaminobutane, which was reacted with 1.25 g of methyl amfenac and the process was repeated. The combined crude products were chromatographed on silica gel eluting with DCM and 0.5% MeOH/DCM. The crude product (**15)** was a yellow crystalline solid weighing 12.3 g which was chromatographed again eluting with 2, 5% acetone/DCM. The product amfenac butylamide-azide (**15**) so obtained was a yellow crystalline solid weighing 8.30 g (77.1%). $^{1}$H NMR (300 MHz, $CDCl_3$): δ 7.55 (d, 2H), 7.45 (d, 1H), 7.41-7.35 (m, 3H), 7.15 (d, 1H), 6.62 (b, 2H), 6.52 (t, 1H), 5.62 (t, 1H), 3.45 (s, 2H), 3.20 (m, 4H), 1.48 (m, 4H). IR (KBr, $cm^{-1}$): 3428, 3318 (N-H, amine); 3260 (N-H, amide) 3081, 2925 (C-H); 2092 (N=N=N); 1634, 1580 (C=O, amide); 1613 (C=O, ketone); 1556 (N-H, amine). MS (m/z, M+H): Calcd. for $C_{19}H_{22}N_5O_2$: 352.17 Found: 352.3 M.p.: 81–83 $^{o}$C.

**Preparation of the amfenac butylamide-amine (13a):**

Compound **15** (970 mg, 2.76 mmol) was dissolved in 10 mL of MeOH. Then 5% $Pd/CaCO_3$ (50 mg) was added and the reaction was evacuated and flushed with hydrogen three times. The reaction was then stirred at room temperature under 1 atm of hydrogen for 24 h. The reaction was then filtered through Celite and the solvent removed *in vacuo* to give the amfenac butylamide-amine (**13a**) as a yellow crystalline solid weighing 900 mg (100%). $^1$H NMR (300 MHz, $CDCl_3$): δ 7.61 (d, 2H), 7.58-7.36 (m, 4H), 7.21 (d, 1H), 6.80 (b, 2H), 6.75 (t, 1H), 6.58 (t, 1H), 3.54 (s, 2H), 3.22 (q, 2H), 2.65 (t, 2H), 1.85 (b, 2H), 1.55 (q, 2H), 1.45 (q, 2H). MS (m/z, M+H): Calcd. for $C_{19}H_{24}N_3O_2$: 326.41 Found: 326.1 IR (KBr, $cm^{-1}$): 3338 (N-H, amine, amide); 2928, 2866 (C-H); 1647 (C=O, amide); 1617 (C=O, ketone); 1559 (N-H, amine). TGA: $T_d$ = 302 $^o$C, $T_m$ = 108.9 $^o$C.

**Preparation of amfenac poly(butylamide-imine) (12a):**

Compound **13a** (500 mg, 15.4 mmol) was heated to 200 $^o$C *in vacuo* while being mechanically stirred for 3 h and then was cooled to room temperature. The resulting product was an amber glass. Yield was quantitative. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.60–6.30 (b, 11H), 3.6-3.0 (b, 6H), 1.80-1.30 (b, 4H). IR (KBr, $cm^{-1}$): No discernible individual absorbances. GPC (THF): $M_w$ = 2.6 kDa, $M_n$ = 1.0 kDa, PDI = 2.6. DSC: $T_g$ = 153 $^o$C; $T_m$ = 231.6 $^o$C.

**Degradation study of the amfenac (butylamide-imine) (13a):**

Compound **13a** (2.5 mg) was dissolved in 1.00 mL of pH 4.0 sodium acetate/acetic acid buffer. An aliquot of a stock solution of cathepsins B and D (50 mg)

(Invitrogen) was added to give an approximate concentration of enzymes of 50 pM. The resulting solution was incubated in an incubator/shaker (Excella E25, New Brunswick Scientific) at 37 $^{o}$C. The solution was analyzed by thin-layer chromatography using 15% MeOH/$CH_2Cl_2$ as eluent. Standard solutions of lactam and amfenac were co-spotted for comparison and the chromatogram visualized using UV (254 nm) light. Samples were analyzed after one day and after six days. On the sixth day, lactam (but not amfenac) was clearly visible.

## 2.5 Degradation Study of PolyAspirin Microspheres Incorporating Sodium Amfenac

Polymeric microspheres have proven to be useful for the delivery of therapeutic agents and are the fabrication form of choice for administration by injection.[29] An alternative method for the delivery of amfenac to the posterior of the eye is to physically incorporate sodium amfenac into microspheres fabricated from PolyAspirin. This approach was accomplished using a w/o/w emulsion technique[29], resulting in microspheres of 2-50 microns in diameter. The microspheres appeared to be round and smooth with some porosity visible. **Figure 8** is a scanning-electron micrograph (SEM) image of these microspheres.

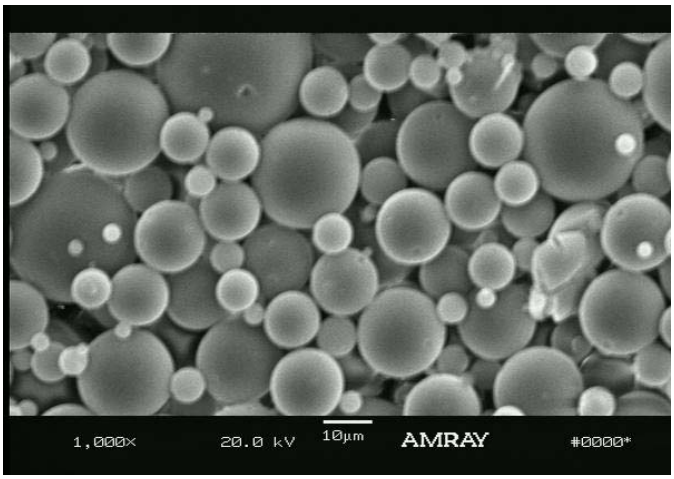



**Figure 8:** SEM image of PolyAspirin microspheres incorporating sodium amfenac.

To determine the amount of amfenac incorporated into the microspheres, the microspheres were completely degraded using NaOH in aqueous methanol and the resulting solution analyzed by liquid-chromatography (LC). The average % incorporation of amfenac in three samples was 11.4%. To determine the drug release rate under physiological conditions, the microspheres were incubated in PBS buffer at pH 7.4 and sodium acetate/acetic acid buffer at pH 4.0 at 37 $^{o}$C and the supernatant analyzed by LC. These results are summarized in **Figure 9**. The minimum solubilities of the three components in each buffer was determined (See **Table 2** in **Experimental**) and found to be far in excess of the concentrations observed during the study.

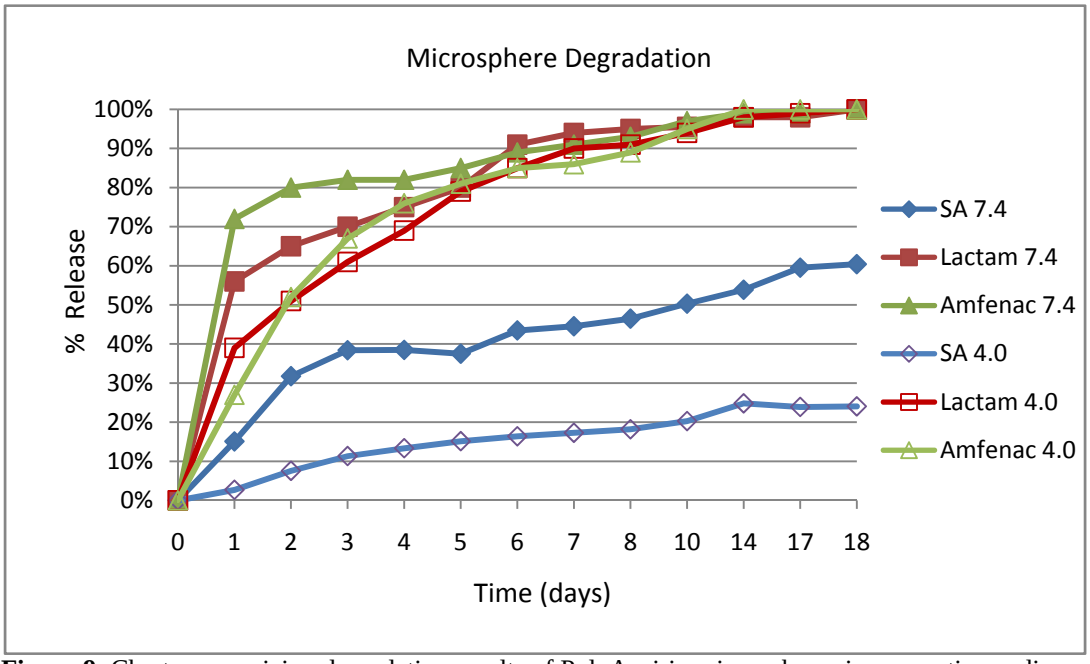



**Figure 9**: Chart summarizing degradation results of PolyAspirin microspheres incorporating sodium amfenac.

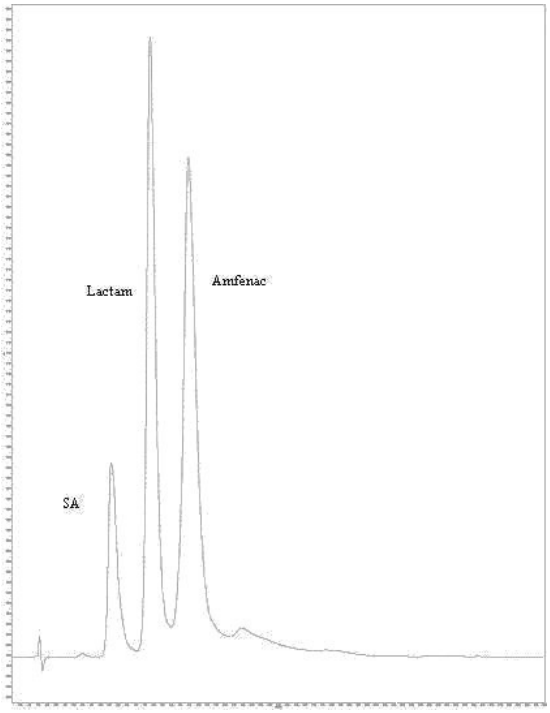



**Figure 10**: Chromatogram showing elution order of SA, lactam (**7**) and amfenac (**1**).

The data summarized in **Figure 9** is the average of three samples at each pH. For SA, the percent release was calculated from the theoretical amount based on the mass of the sample. For the lactam and amfenac, the percent release was based on the total amount released as determined by LC. The study was concluded when the levels of lactam and amfenac became undetectable. The LC solvent system used was 1:1 MeOH/water/1% AcOH. SA, lactam and amfenac had retention times of 5.7, 7.8 and 9.7 minutes, respectively (**Figure 10**).

The data reveals that microsphere degradation is more rapid at pH 7.4 than 4.0, 60% after eighteen days versus 25%, as indicated by SA release. This result is in accord with previous results obtained with PolyAspirin.[4]

Drug (lactam and amfenac) release from the microspheres is also more rapid at pH 7.4 than 4.0, approximately 70% at pH 7.4 versus approximately 28% at pH 4.0 over one day for amfenac. One reason is that amfenac exists as the salt at pH 7.4; at pH 4.0 it exists as the acid. The salt is more hydrophilic than the acid and is more readily diffused into the aqueous medium.

However, it appears that drug release is considerably faster than microsphere degradation. This finding indicates that drug is not incorporated into the polymer matrix, but resides largely on the surface. This result is not surprising considering that sodium amfenac is very hydrophilic. Thus, incorporating sodium amfenac into PolyAspirin microspheres is not a feasible method of sustained release for this drug, especially as the treatment of posterior uveitis requires a release time of at least 4-6 weeks. A better alternative would be nepafenac, which is considerably less hydrophilic than sodium

amfenac, and should be more effectively incorporated into the microspheres, leading to a longer release time.

## 2.5a Experimental

**Microsphere preparation:** *Carried out by M. Ouimet*

*Sodium amfenac (50.0 mg) was dissolved in 1 mL of deionized water. This solution was added dropwise to a 3.0 mL solution of salicylic acid-adipic polymer (166 mg/mL) in DCM while homogenizing at a speed of "4" with a IKA Ultra-Turrax T8 homogenizer. After all the sodium amfenac solution had been added, the water-in-oil suspension was further homogenized for three additional minutes. This suspension was then added dropwise to 100 mL of 1% aqueous polyvinyl alcohol while homogenizing at speed "3". After addition was complete, homogenization was continued for three minutes. This suspension was then mechanically stirred at room temperature for 2.5 h. It was then divided between three 50 mL centrifugation tubes and centrifuged at 3000 rpm for 10 minutes with a Hettich EBA12 centrifuge. The supernatant was decanted and 35 mL of pH 2.0 hydrochloric acid added. The tubes were shaken briefly and centrifuged for five minutes at 3000 rpm. The acid wash was repeated five times (until foam no longer formed). The (three) samples were then frozen at -78 $^{o}$C and lyophilized for 48 h (Labconco Freezone 4.5 lyophilizer). The total weight of microspheres obtained was 407 mg (74%).*

**Microsphere degradation with sodium hydroxide:**

Three samples of microspheres (3.9 mg, 3.2 mg and 5.7 mg) were dissolved in 0.2 mL of 1:1 1N NaOH/MeOH and stirred at room temperature overnight. The resulting solutions were diluted to 2.0 mL with MeOH, filtered through a 0.45 μm Teflon filter and analyzed by LC. The results are shown in **Table 1**. The average % incorporation was 11.4.

| Weight of Sample (mg) | Weight of Sodium Amfenac (μg) | % Incorporation |
|---|---|---|
| 3.9 | 715 | 18 |
| 3.2 | 313 | 9.8 |
| 5.7 | 370 | 6.5 |

**Table 1**: NaOH degradation and % incorporation of sodium amfenac into PolyAspirin microspheres.

**Solubility of SA, lactam and amfenac:**

A 3-5 mg sample of each component was stirred in 2.0 mL of PBS 7.4 and sodium acetate/acetic acid pH 4.0 buffers for 24 h, the solutions filtered through a 0.45 μm Teflon filter and subjected to LC analysis. These results are shown in **Table 2**.

| Buffer/pH | SA Solubility (μg/mL) | Lactam Solubility (μg/mL) | Amfenac Solubility ( μg/mL) |
|---|---|---|---|
| PBS 7.4 | 2752 | 32 | >1450 |
| NaOAc/AcOH 4.0 | >1600 | 35 | >81 |

**Table 2**: Solubility of SA, lactam and amfenac in PBS 7.4 and sodium acetate/acetic acid pH 4.0 buffers at room temperature.

**Microsphere degradation at pHs 7.4 and 4.0:**

Three separate samples of microspheres (4.7 mg, 4.8 mg and 5.9 mg) were placed in 10 mL plastic centrifuge tubes followed by 10.0 mL of pH 7.4 PBS buffer. Another three samples of microspheres (5.3 mg, 4.9 mg and 4.6 mg) were added to 10 mL centrifuge tubes followed by 10.0 mL of pH 4.0 sodium acetate/acetic acid buffer. The tubes were placed in an incubator/shaker (Excella E25, New Brunswick Scientific) at 37 $^{o}$C. After twenty four hours, the tubes were centrifuged for 10 minutes at 5000 rpm. Supernatant (5.00 mL) was then removed via syringe from each tube and replaced with fresh buffer. The tubes were then vortexed for five seconds and placed back into the incubator. The removed supernatants were filtered through a 0.45 μm Teflon filter and analyzed by LC. The pH of each sample was checked with pH paper prior to analysis. No change in pH was observed with any sample.

**LC calibration curves for SA, lactam and amfenac:**

Standard solutions of each compound were prepared at concentrations of 50, 500, 1000 and 2000 μg/mL by successive dilution in 1:1 MeOH/water/1% AcOH, which is the eluent used for the chromatographic analysis. The concentration of each solution was determined in duplicate. The results are summarized in **Figures 11 – 13** for salicylic acid, lactam and amfenac, respectively.

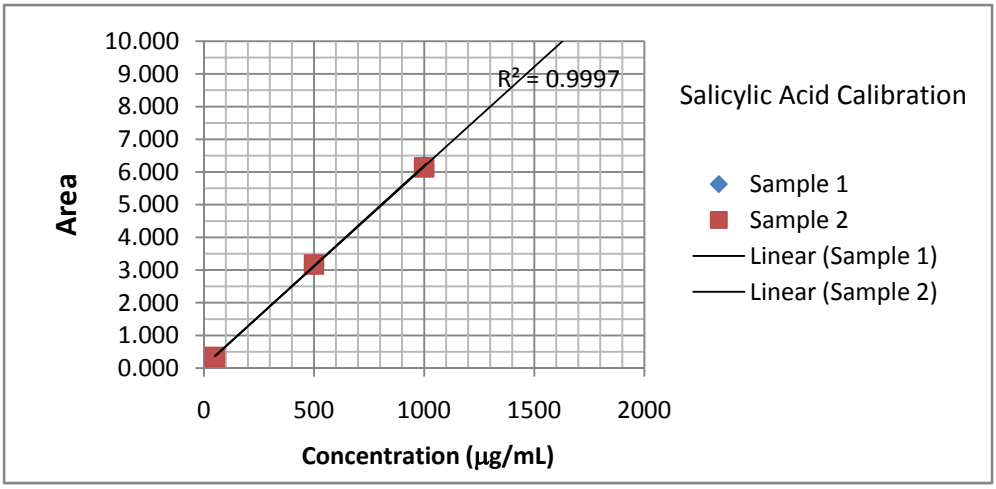



**Figure 11:** Calibration curve for salicylic acid.

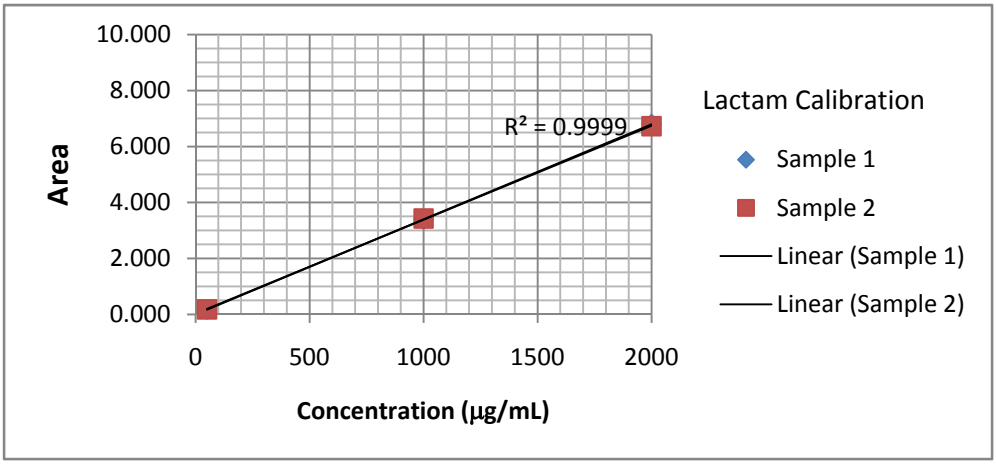



**Figure 12**: Calibration curve for the lactam **(7).**

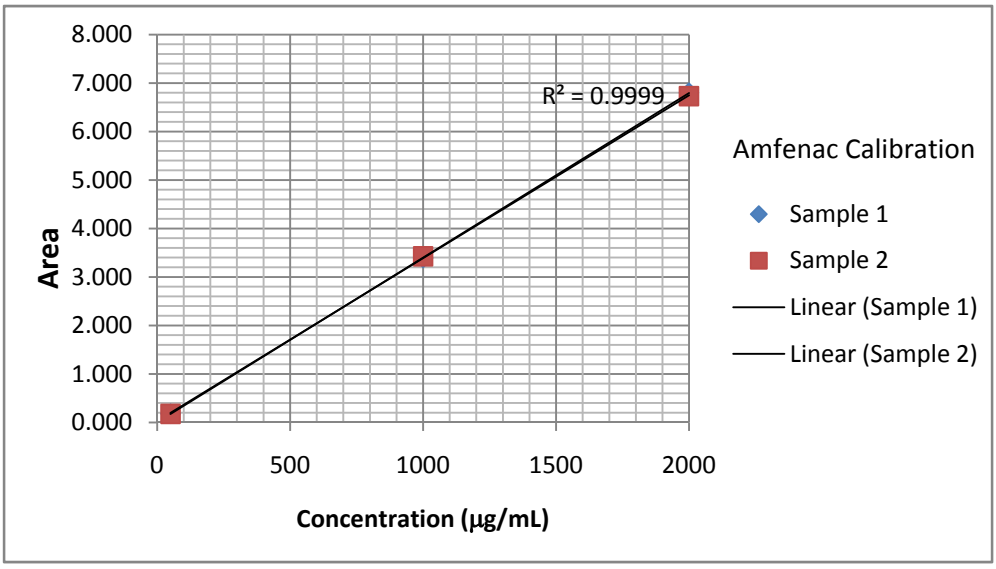



**Figure 13**: Calibration curve for amfenac **(1).**

# 3. Polymers for the Sustained Release of a Nutlin Analog for the Treatment of Brain Cancer

## 3.1 Introduction

The past twenty years have seen significant advances in the diagnosis and treatment of cancer, resulting in an overall improvement in survival rates of cancer patients.[30] However, brain cancers (gliomas) remain one of the most deadly of cancers, with five year survival rates of less than twenty percent for younger patients (<40 years old) and less than five percent for older (>40) patients.[31, 32]

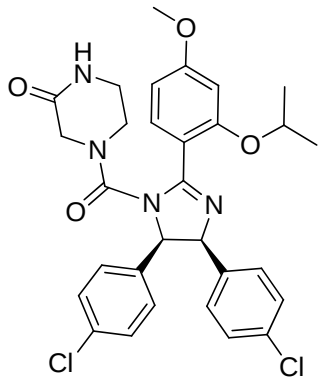
Various reasons for the high mortality rate of gliomas are proposed. These include the impenetrability of the blood-brain barrier[31], rendering systemic administration of anti-cancer drugs impractical, as very high doses would be necessary to overcome the blood-brain barrier and anticancer drugs are typically highly toxic substances to normal as well as cancer cells.[31] Also, many brain tumors rapidly develop resistance to chemotherapeutic agents.[31]

Another major reason for the high mortality rate of gliomas is the high incidence of recurrence due to the difficulty in complete surgical removal of the glioma[31], a consequence of the characteristic of gliomas to permeate the surrounding tissue. One method of mitigating recurrence is to implant polymers incorporating anticancer agents into the excision void after surgery. The drug is released slowly over time and localization mitigates toxicity to the surrounding tissue. The Gliadel Wafer™ is a polyanhydride incorporating the anticancer drug carmustine.[33] Fabricated into disks, it is

implanted after surgery to kill remaining glioma cells. Carmustine, however, is a highly toxic drug and has serious side-effects.[31] Thus, it is desirable to employ less toxic agents. One recently discovered class of anticancer drugs is the nutlins, developed by Hoffman-LaRoche, Inc. The nutlins belong to a class of drugs known as MDM2 inhibitors.[34]

The p53 protein has been identified as a main regulator of cell division and apoptosis (programmed cell death) and its function is impaired in many cancers.[35,36] In some cancers, p53 is mutated into an inactive form. In other cancers, the main regulatory protein of p53, MDM2, is overexpressed.[36] MDM2 facilitates extracellular transport of p53 and also catalyzes its lysosomal degradation.[36] Thus, MDM2 inhibition has emerged as a promising therapeutic approach in cancer treatment.[36]

The chemical structure of nutlin 3a, one of the first of the nutlin class of agents to be identified, is shown in **Figure 14**. Nutlin 3a exhibits an $IC_{50}$ of .09 nM in an MDM2 inhibition assay.[34]




**Nutlin 3a**
**$IC_{50}$ = .09 μM**

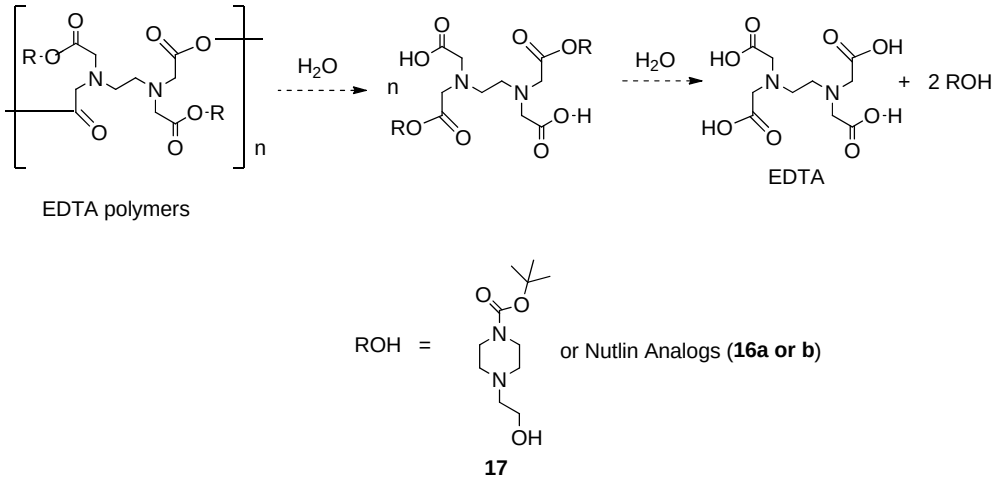
**Figure 14**: Chemical structure of nutlin 3a.

Henceforth will be described efforts to incorporate a nutlin analog (**16**) into a polymer for localized and sustained release for brain cancer treatment. This polymer may be fabricated into disks to be implanted into the excision void after surgery to prevent recurrence.

## 3.2 Attempted Synthesis of an EDTA-based Polyanhydride Incorporating a Nutlin Analog

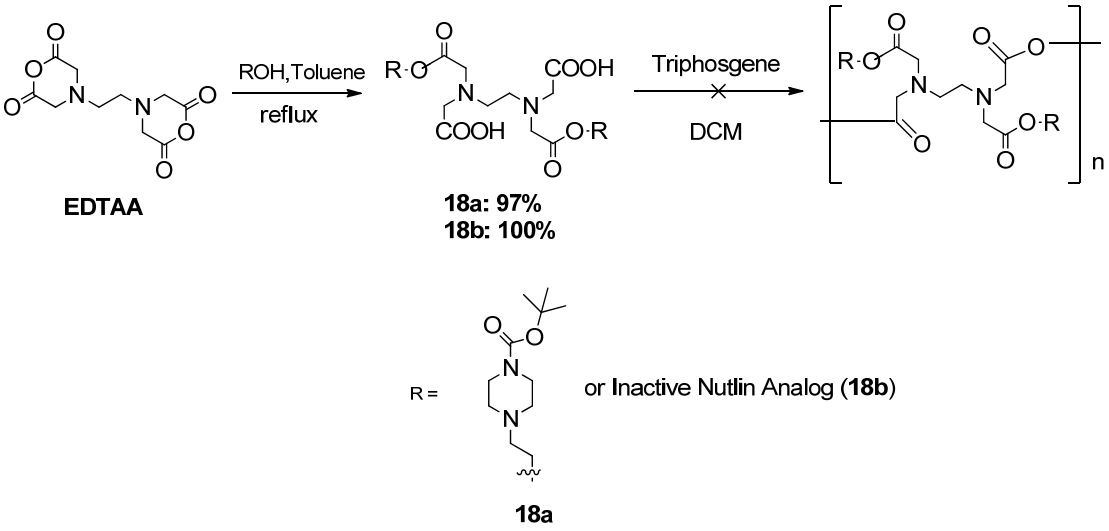
The nutlin analog has one chemical functional group suitable for chemical incorporation into a polymer: a hydroxyl group. Thus, the "pendant attachment" (**Fig. 2B**) approach is the only applicable method of chemical incorporation. To take advantage of its known safety profile, symmetrical structure and antimicrobial properties, ethylenediaminetetracetic acid (EDTA) was utilized for the polymer backbone.[37]

The nutlin compounds are structurally complex molecules, bearing two chiral centers, and therefore presumably difficult to synthesize. Thus, they are expensive substances such that a model alcohol was employed to establish the methodology for the polymer synthesis. The model alcohol was *t*-butylcarboxypiperazineethanol (**17, Scheme 8**). Also, the inactive enantiomer (**16b**) was utilized whenever possible to spare the active enantiomer (**16a**). The structures of the proposed polymers and their degradation products are depicted in **Scheme 8**. The structures of the nutlin analogs are not shown for proprietary reasons, nor is any spectroscopic data for the compounds derived from them.




**Scheme 8**: Chemical structurs of the proposed polymers and their degradation products.

The synthesis of the polymers is depicted in **Scheme 9**. It was anticipated that the alcohols could be reacted with EDTA dianhydride (EDTAA) in a suitable solvent to give the diacids of the model alcohol (**18a**) as well as the inactive enantiomer (**18b**), which could then be polymerized using triphosgene. The presence of the tertiary amine in the model alcohol would obviate the addition of a base (e.g. triethylamine), resulting in the hydrochloride salt of the polymer. The free base could be obtained, presumably, by using the sodium salt of the diacid.




**Scheme 9:** Attempted synthesis of the proposed polymers derived from EDTA.

The model alcohol (**17**) reacted smoothly with the commercially available EDTAA in refluxing toluene to give the desired diester-diacid (**18a**) in 97% yield after two hours. Likewise, the inactive nutlin analog (**16b**) also reacted smoothly with the anhydride in refluxing toluene, but the reaction took six to eight hours instead of two, presumably due to the increased steric hindrance of the bulkier molecule. Nonetheless, the desired EDTA diester-diacid of the inactive nutlin analog (**18b**) was obtained in 89-100% yield in a very clean reaction. Notably, this reaction had to be run under strictly anaerobic conditions as the nutlin analog is readily oxidized to the imidazole, which appears as a blue fluorescent spot just above the nutlin analog on thin-layer chromatography. This observation was substantiated by our collaborators at Hoffmann-LaRoche.

Unfortunately, all attempts to effect polymerization of either diacid utilizing triphosgene, carbonyldiimidazole or ethyl chloroformate were unsuccessful, yielding only low molecular weight oligomers. GPC and MALDI (performed by collaborators at Hoffmann-LaRoche) indicated that trimers were formed. To determine if the tertiary amine in the model alcohol was responsible for the failure, the diethyl ester of EDTA was prepared[38] and the polymerization attempted, but this compound failed to react. Finally, polymerization of the disodium salt of diethyl EDTA was attempted, but this too failed to react. Thus, it appeared that a polyanhydride derived from EDTA was not possible and alternate solutions were pursued.

## 3.2a Experimental

**Preparation of the EDTA-model alcohol diacid (18a):**

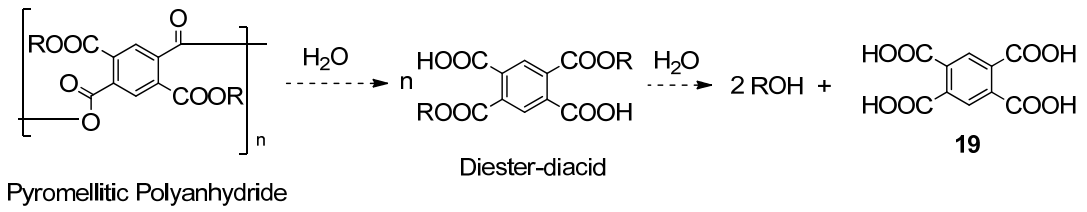
EDTAA (556 mg, 2.17 mmol) and N-*t*-butylcarboxypiperazineethanol (**17**) (1.00 g, 4.34 mmol) were suspended in 10 mL of dry toluene and refluxed under argon for 2 h. The reaction was then cooled to room temperature, filtered and the toluene evaporated *in vacuo* to give **18a** as a brown foam weighing 1.52 g (97.3 %). $^{1}$H NMR (300 MHz, DMSO-$d_6$): δ 13.0 (b, 2H), 4.15 (t, 4H), 3.58 (s, 4H), 3.45 (s, 4H), 3.29 (t, 8H), 2.76 (s, 4H), 2.55 (t, 4H), 2.36 (t, 8H), 1.39 (s, 18H). MS (m/z, M+H): Calcd. for $C_{32}H_{57}N_6O_{12}$: 717.4 Found: 717.4. IR (KBr, $cm^{-1}$): 3455 (OH, carboxylic acid); 2977 (C-H); 1743 (C=O, ester); 1695 (C=O, carboxylic acid); 1631 (C=O, urethane).

**Preparation of the EDTA diacid of the inactive nutlin analog (18b):**

The inactive nutlin analog (**16b**) (681 mg, 1.11 mmol) and EDTAA (143 mg, 0.557 mmol) were dissolved/suspended in dry toluene and refluxed under argon for 16 h. The reaction was then cooled, filtered and the solvent evaporated *in vacuo*. The product was a tan solid weighing 880 mg (100%). (Spectroscopic data has been omitted for proprietary reasons).

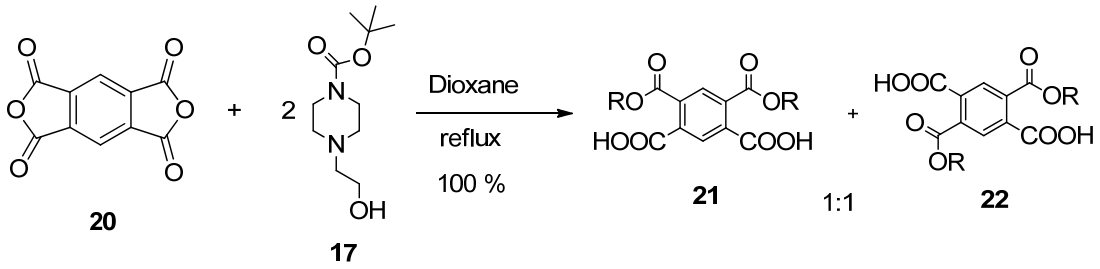
## 3.3 Efforts to Synthesize a Pyromellitic Acid-based Polyanhydride Incorporating a Nutlin Analog

As a symmetrical alternative to EDTA, pyromellitic acid was used, as esters derived from it have already been shown to be precursors to polyanhydrides.[39] The proposed polymers and their degradation products are depicted in **Scheme 10**.



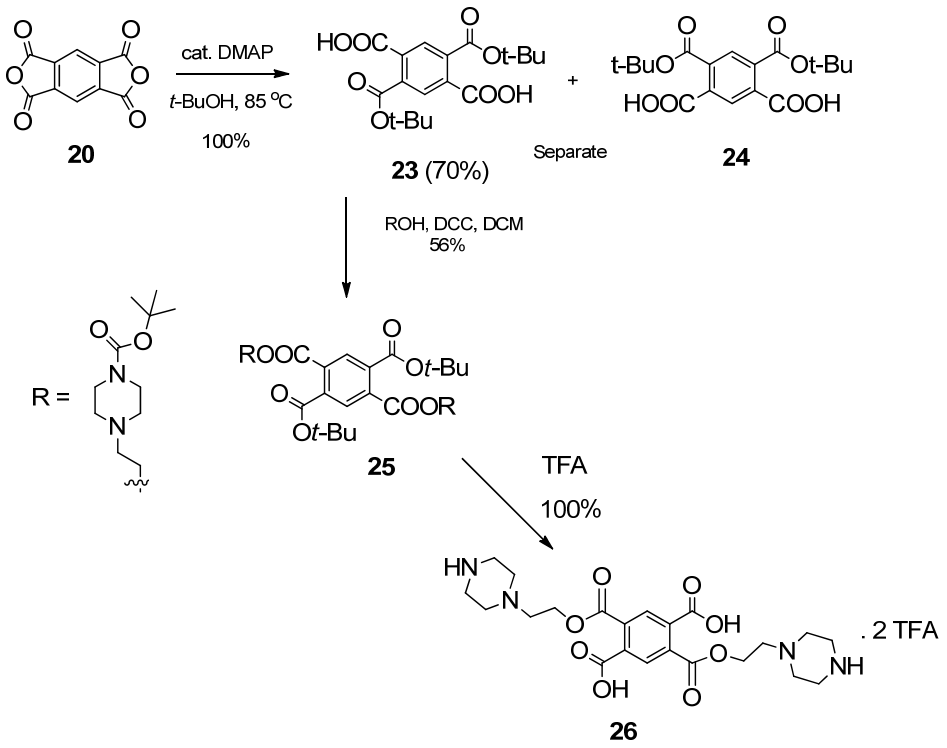

**Scheme 10**: Proposed polymers based on pyromellitic acid and their expected degradation products, alcohol and pyromellitic acid (**19**).

The synthetic methodology would be established using the model alcohol and then extended to the nutlin analog as before. The first attempt to prepare the diacid precursor to the polyanhydride was reaction of pyromellitic anhydride (**20**) with the model alcohol directly (**Scheme 11**) as performed with EDTAA. As expected from the literature, this reaction gave a 1:1 mixture of 1,3 (**21**) and 1,4 (**22**) diacids.[40]




**Scheme 11**: First attempted synthesis of the model alcohol pyromellitate diacids.

Unfortunately, **21** and **22** proved impossible to separate, either by crystallization or chromatography. This necessitated the approach depicted in **Scheme 12**. This approach entails reaction of pyromellitic anhydride (**20**) with *t*-BuOH to give the diesters, which will then be separated and reacted with the model alcohol (**17**), followed by removal of the *t*-butyl groups to give the desired diacids (**21** and **22**).




**Scheme 12**: Alternative approach to pyromellitic acid-based polymers.

The di-*t*-butyl esters (**23** and **24**) are known compounds which have been previously synthesized by a different method.[40] The method shown, using *t*-BuOH and dimethylaminopyridine (DMAP) at 85 $^{o}$C, is novel and gives a quantitative yield of a 1:1 mixture of the respective isomers. The pure para isomer (**23**) was obtained by trituration with 95% aqueous ethanol in 70% theoretical yield based on the 1:1 mixture. Interestingly, it was found that **23** and **24** are thermally unstable above 110 $^{o}$C, although TGA analysis indicated a $T_d$ of 174 $^{o}$C.

Compound **23** was then reacted with the model alcohol using dicyclohexylcarbodiimide (DCC) in dichloromethane (DCM) to give the desired tetraester (**25**) in 56% yield after chromatography. According to NMR data, the crude

yield of **25** is very high, nearly quantitative, implying that considerable decomposition occurs upon chromatography on silica gel.

The next step involved removal of the *t*-butyl groups and treatment of tetraester **25** with trifluoroacetic acid removed all the *t*-butyl groups, including those on the alcohol portion of the molecule, yielding diacid **26**. Clearly, an alternative protecting group is warranted as the nutlin analog is a urea, not a urethane, and a urea will be inert to trifluoroacetic acid.

In future work, the *t*-butyl urethane can be replaced with a different protecting group (e.g. a urea), whereby the removal of the pyromellitate *t*-butyl groups will be selective, and the resulting diacid can be polymerized utilizing triphosgene.[41]

## 3.3a Experimental

**Preparation of 1,4-*t*-butylpyromellitic ester (23):**

Pyromellitic anhydride (10.0 g, 45.8 mmol) was refluxed in 50 mL of acetic anhydride for 4 h. Then 30 mL of the anhydride was distilled off and the resulting slurry cooled to room temperature, filtered and the filtrant washed with ethyl acetate. The purified pyromellitic anhydride so obtained was a tan granular solid weighing 7.90 g. This mixture was suspended in 50 mL of anhydrous *t*-BuOH, 50 mg of DMAP were added, and the reaction stirred overnight at 85 $^{o}$C. The solvent was then removed *in vacuo* at 90 $^{o}$C and the residue triturated with 25 mL of 95% EtOH overnight. The resulting slurry was then filtered, washed with 95% EtOH and dried at 80 $^{o}$C *in vacuo*. The 1,4-*t*-butylpyromellitate so obtained was a white solid weighing 4.67 g (70.5%). $^{1}$H

NMR (300 MHz, DMSO-$d_6$): ð 7.88 (s, 2H), 1.52 (s, 18H). MS (m/z, M-H): Calcd. for $C_{18}H_{21}O_8$: 365.13 Found: 365.1 IR (KBr, $cm^{-1}$): 2980 (OH, carboxylic acid); 2671, 2564 (C-H); 1727 (C=O, ester); 1695 (C=O, carboxylic acid). TGA: $T_d$ = 174 $^oC$.

**Preparation of the di-*t*-butyl–di-(N-*t*-butylcarboxypiperazineethanol) pyromellitate ester (25):**

Compound **23** (1.00 g, 2.73 mmol) was dissolved in 10 mL of dry DCM followed by 5.46 mL (2.00 eq) of 1.0 M DCC/DCM and 50 mg of DMAP. After stirring at room temperature overnight, the solvent was removed *in vacuo* and the residue extracted with 2 x 50 mL of ethyl ether. The combined extracts were filtered through Celite and evaporated *in vacuo* to give the crude product as a white solid weighing 2.2 g. This product was chromatographed on silica gel eluting with 1, 2% MeOH/DCM. The product (**25**) was a white crystalline solid weighing 1.2 g (56%). $^1H$ NMR (300 MHz, $CDCl_3$): ð 7.95 (s, 2H), 4.47 (t, 4H), 3.43 (t, 8H), 2.75 (t, 4H), 2.48 (t, 8H), 1.58 (s, 18H), 1.45 (s, 18H). MS (m/z, M+H): Calcd. for $C_{40}H_{63}N_4O_{12}$: 791.44 Found: 791.3. IR (KBr, $cm^{-1}$): 3000-2750 (C-H); 1737 (C=O, ester); 1719 (C=O, ester); 1690 (C=O, urethane). M.p.: 134-136 $^oC$.

**Preparation of the di-piperazineethanol pyromellitate diacid (26):**

Compound **25** (100 mg, 1.25 mmol) was dissolved in 1.00 mL of trifluoroacetic acid and stirred at room temperature for 1 h. The solvent was removed *in vacuo* and the residue azeotroped *in vacuo* with 3x5 mL of toluene. The product was a light brown solid. $^1H$ NMR (300 MHz, DMSO-$d_6$): δ 8.90 (b, 2H), 8.05 (s, 2H), 6.30 (b, 4H), 6.47 (t, 4H), 3.22 (b, 8H), 3.15 (b, 4H), 3.02 (b, 8H).

# A1. Appendix 1: Materials and Methods

## A1.1 Materials

All solvents and reagents were purchased from Sigma-Aldrich and used as obtained without any further purification with the exception of 1,4–dibromobutane, which was purchased from Oakwood Products, Inc. and used as is. N-*t*-butylcarboxypiperazineethanol and both enantiomers of the nutlin analog (**16a** and **16b**) were obtained from Hoffmann-LaRoche, Inc. Pyromellitic anhydride was purified as described in the experimental procedure.

## A1.2 Analyses

### *Spectroscopic Analysis*

$^{1}H$ and $^{13}C$ NMR spectra were obtained on a Varian 300 MHz instrument (Palo Alto, CA) using TMS as internal standard. IR spectra were obtained on an AVATAR 360 FT-IR from Thermo Nicolet (Shelton, CT). Samples were prepared as KBr pellets or solvent-cast onto NaCl plates. Mass spectra were obtained on a Finnigan LCQ DUO from Thermo Quest (San Jose, CA).

### *Thin-Layer Chromatographic Analysis*

Thin-layer chromatographic analysis was carried out on Whatman glass-backed silica gel plates of 250 μm thickness and 60 Å pore size with fluorescent indicator. Spots were visualized using UV light or iodine.

## *Thermal Analysis*

Melting points ($T_m$) and glass-transition temperatures ($T_g$s) were determined on a Melt-Temp apparatus from Laboratory Devices (Cambridge, MA) or through differential-scanning calorimetry (DSC). DSC was performed using a Thermal Advantage (TA) DSC Q200 running on an IBM ThinkCentre computer equipped with TA Universal Analysis software for data collection and processing. Samples (5-8 mg) were heated under nitrogen from -10 ˚C to 250 ˚C at a rate of 10˚C/min. A minimum of two heating/cooling cycles were used. TA Instruments Universal Analysis 2000 software, version 4.5A was used to analyze the data. Data points were taken from the onset of the thermocline.

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer TGA7 running Pyris software on a Dell OptiPlex computer employing a Perkin-Elmer TAC 7/DX controller. Samples (3-5 mg) were heated under nitrogen from 0 $^{o}$C to 600 $^{o}$C at a rate of 20 $^{o}$C/minute.

## *Size and Morphology of Microspheres*

Size and morphology of the microspheres were determined using SEM. Images were obtained using an AMRAY-1830I microscope (AMRAY Inc.) after coating the samples with Au/Pd using a sputter coater (SCD 004, Blazers Union Limited).

## *Molecular Weight*

Gel permeation chromatography (GPC) was used to determine the $M_w$ of polymers. A Perkin-Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor were used. A Dell

OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software was utilized for data collection and control. The connection between the LC system and the computer was made using a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Samples were dissolved in dichloromethane (10 mg/mL) and filtered through 0.45 μm Teflon syringe filters (Whatman, Clifton, NJ) prior to elution through a Jordi divinylbenzene mixed-bed GPC column (7.8 x 300 mm) (Alltech Associates, Deerfield, IL) at a rate of 1 mL/min for a total run time of 30 min eluting with dichloromethane. $M_w$ was calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

GPC analysis was also performed on a Water Stryagel 3 THF column. The Waters LC system (Milford, MA) was equipped with a Perkin-Elmer 2414 refractive index detector, a 1515 isocratic HPLC pump and 717Plus autosampler. An IBM ThinkCentre computer with Waters Breeze Version 3.30 software installed was used for the collection and processing of data. Samples were prepared at a concentration of 10-20 mg/mL in THF and filtered using 0.45 μm Teflon filter. The flow rate was 1 mL/minute. $M_w$ was calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

### *Liquid-Chromatographic Analysis*

Liquid-chromatographic analyses were performed on a Waters 2695 Separations Module employing a Waters 2487 Dual Wavelength Detector and a Waters Symmetry $C_{18}$-5 μm 4.6 x 150 mm column. The hardware was controlled by an IBM ThinkCentre computer employing Empower software. The mobile phase was 1:1 methanol-water with

1% acetic acid at a flow rate of 1 mL/minute. The detection wavelength was 245 nm. Calibration was done with data points of 50, 500, 1000 and 2000 μg/mL.

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