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Short Note

2-((Diphenylmethylene)amino)ethyl *N*-(Cyclohexyl)carbamate

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Abstract: Lipid-like nanoparticles (LLNPs) have been shown to be an effective encapsulation and delivery tool for therapeutic molecules. While the preclinical development of lipid nanoparticle formulations has been of paramount importance, next-generation LLNPs present an opportunity of enhanced biocompatibility. With the change in amido functionality as part of the core backbone, our target, carbamate functionality within the LLNP core scaffold, was realized upon reaction of a protected amino alcohol onto the isocyanate generated in situ via a Curtius rearrangement. The single-step assembly of carbamate functionality starting from cyclohexane carboxylic acid in the presence of diphenylphosphoryl azide (DPPA) exceeds the metrics set forth for the rapid installment and enhanced biodegradability of next-generation lipid-like nanoparticles.

Keywords: Curtius rearrangement; carbamate functionality; lipid-like nanoparticles (LLNPs); synthetic design

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1. Introduction

What can arguably be considered one of the most revolutionary platforms for the delivery of therapeutic molecules is lipid-like nanoparticles (LLNPs) [1]. Composed of two structural motifs, a core backbone covalently linked to hydrophilic tendrils, they, in combination, determine the functionality and performance in drug delivery [2].

The long chains serve to both encapsulate and protect the drug while targeting specific cells or tissues because of the mimicking properties of the lipid-based materials employed. Furthermore, the size, which can be engineered and ranges from twenty to two hundred nanometers, is small enough to enter cells but large enough to avoid renal clearance. Collectively, the delivery mechanism serves as a powerful tool for drug delivery, as recently witnessed with the Pfizer-BioNTech and Moderna COVID-19 vaccines [1].

Among the core backbones employed, 1,3,5-benzene tricarboxylic acid (trimesic acid) has garnered significant interest due to its unique structural and chemical properties. As a rigid, aromatic, and symmetrically trifunctional molecule, 1,3,5-benzene tricarboxylic acid serves as a versatile backbone for constructing LLNPs with precise and enhanced functional properties [3].

The incorporation of a cyclic trifunctionalized core scaffold (Figure 1) offers several advantages in nanoparticle design especially when considering biodegradability. The three carboxylic acid derivatives provide a robust foundation for chemical modifications, enabling the conjugation of diverse lipid-like arms through amide, ester, or carbamate linkages [4,5].

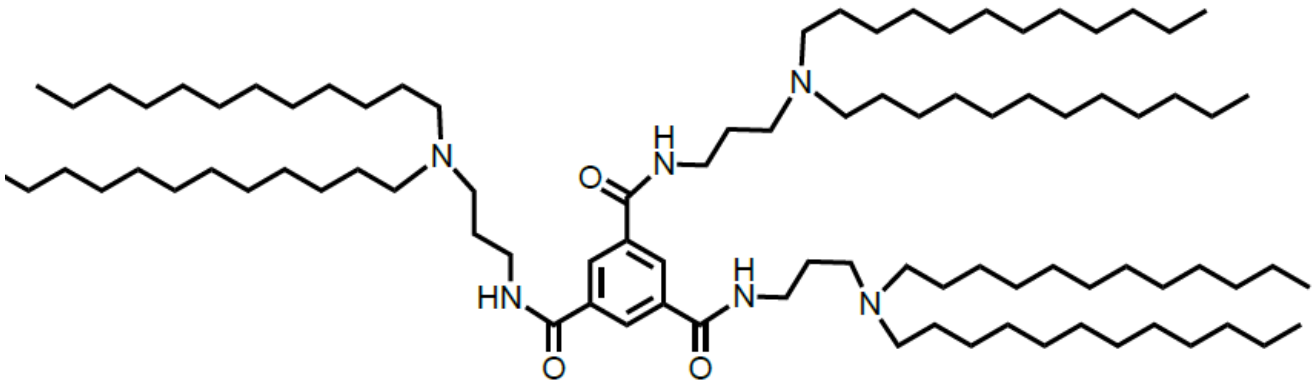
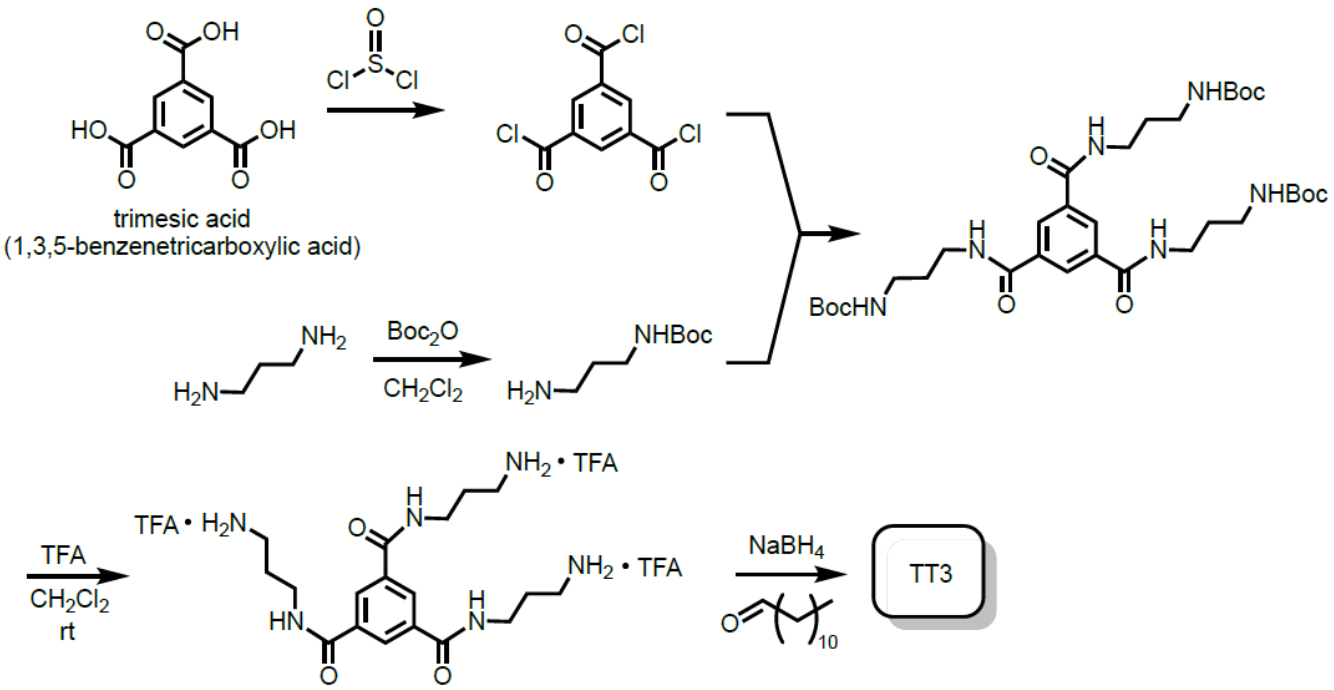


Figure 1. N^1,N^3,N^5-Tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3).

2. Background

By virtue of the D_3h point group, TT3's assembly exploits symmetry-driven uniformity (Scheme 1). The LLNP can be prepared in not 15 but 5 functional group transformations, making this system an ideal candidate for studies focusing on next-generation core backbones. The tricarboxamide functionality stemming from the benzene core bound to dodecyl chains using aminopropyl linkers witnessed TT3's successful role with the delivery of mRNA molecules encoding human factor IX [6], CRISPR–Cas9 [7], and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens [8].



Scheme 1. Synthesis of TT3.

The key synthetic step in TT3's assembly involves a reductive amination of the deprotected BOC-amines with dodecylaldehyde in the presence of sodium triacetoxyborohydride [6]. Formation of the protected amines began with the conversion of trimesic acid to the corresponding thionyl chloride. Once generated, this intermediate efficiently yielded the protected triscarboxamide upon condensation with a BOC-protected 1,3-propanediamine.

When focusing on biodegradability, however, an area yet to be explored using the core backbone found with TT3 is changes to the carboxylic acid derivative. As stated above, a key metric with nanoparticle design is not only drug delivery but biodegradation; hence,

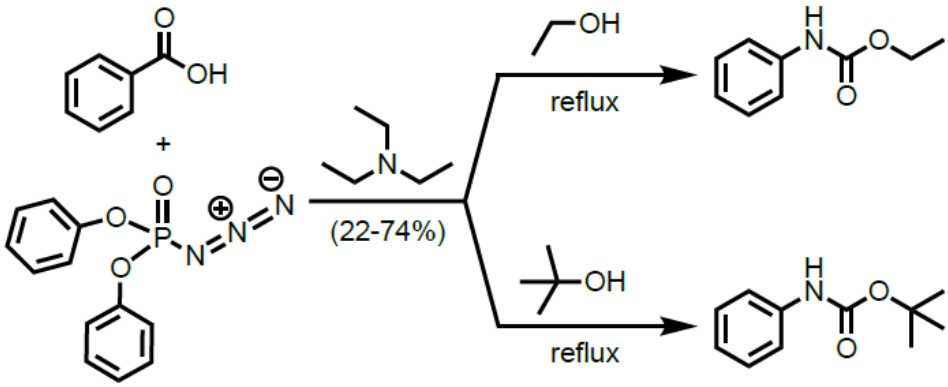
our focus was to explore the assembly of TT3 analogs bearing not amide but carbamate functionality. The change in functionality offers a clear advantage when considering biodegradability, which for this system would occur via hydrolysis or enzymatic action [4].

3. Results and Discussion

In order to achieve our goals of (1) assembling a series of carboxylic acid derivatives and (2) using a unified platform starting from carboxylic acid functionality, we elected to explore the Curtius rearrangement using, as our model system, benzoic acid. The Curtius rearrangement is a well-established transformation used to convert carboxylic acids via their acyl azide to isocyanates [9]. What is unique and addresses both of our goals is that upon changing the reaction workup, the isocyanates yield carbamates when treated with an alcohol, and urea when trapped with an amine. Both transformation and model system are ideal as the core backbone of TT3 bears amide functionality bound to an aromatic scaffold.

Schemes 2 and 3 offer a summary of the results obtained when working with our model system, benzoic acid, as part of the course we offered in the Spring term 2024. While pleased in the outcome as it relates to product formation and use of *t*-butyl alcohol and ethanol as trapping agents, the yields, interestingly, matched the experience of the nine students involved in the research project (CURE) and very much aligned with the highs and lows often witnessed when conducting research [10].

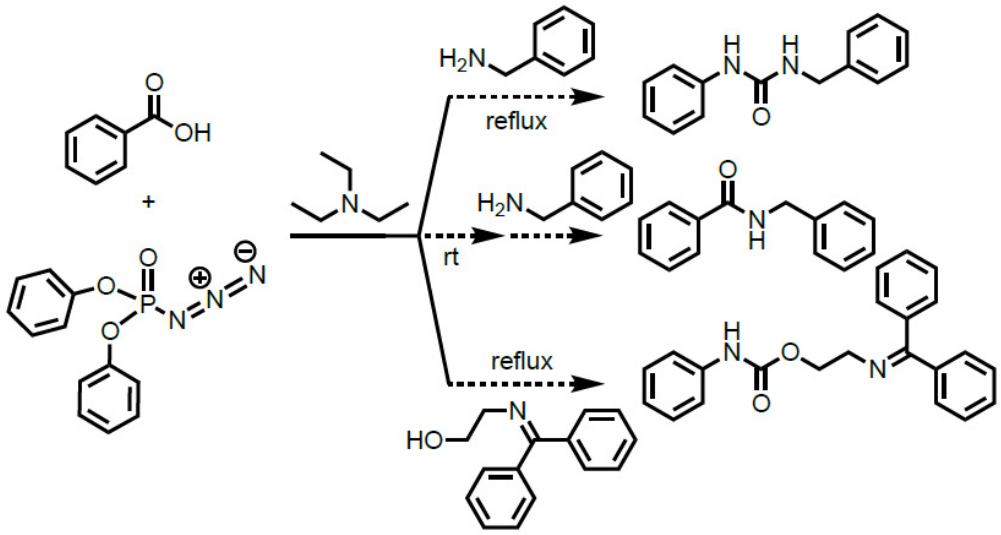
While encouraged with the formation of carbamate functionality when working with an excess of trapping agent (Scheme 2), changes to nucleophile (benzyl amine (top bracket)), temperature (amide formation (middle bracket)), and equivalency (stoichiometric quantities of the trapping agent (bottom bracket)) resulted in, unfortunately, no observable product formation (Scheme 3).



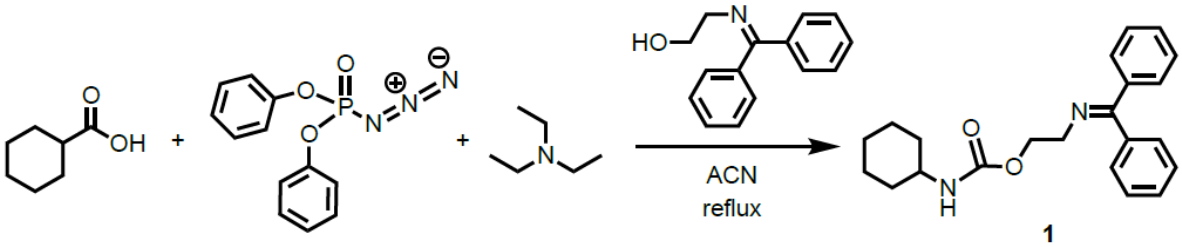
Scheme 2. Formation of *t*-butyl and ethyl carbamates.

What is important to note is that for each of the systems explored in Scheme 3, stoichiometric, not excessive quantities of trapping agent were employed. Furthermore, as the focus of this study was the installment of carbamate functionality and not the scope, we elected to change benzoic acid to the less reactive cyclohexane carboxylic acid (Scheme 4).

We were pleased to see that reaction of cyclohexane carboxylic acid with diphenylphosphoryl azide (DPPA) in the presence of triethylamine resulted in the formation of carbamate **1** when trapped with stoichiometric quantities of the Schiff base 2-((diphenylmethylene)amino) ethanol [11,12].



Scheme 3. Unsuccessful attempts in formation of urea, amide, and carbamate functionality.



Scheme 4. Formation of carbamate **1**.

This result, unoptimized, was important for several reasons. First, the Schiff base derived from 2-aminoethanol would serve as the ideal platform from which condensation of alkyl aldehydes in the presence of sodium triacetoxyborohydride would furnish the lipophilic residues present in TT3. Second, while we feel that use of (1) commercially available phenyl isocyanate with stoichiometric quantities of the Schiff base or (2) excess quantities of the Schiff base when working with benzoic acid and DPPA would result in the desired product formation, both would detract from the overarching goal of a unified, efficient, and atom-economic assembly of next-generation LLNPs bearing carbamate functionality. Third, the one-step installment of carbamate functionality, which avoids the need to work with the far more labile acyl azide, reduces the number of synthetic steps by half when compared to how the amide functionality is installed with TT3; hence, this provides an opportunity to explore next-generation lipid-like nanoparticles using the Curtius rearrangement as the key step in furnishing enhanced biodegradable functionality as part of the core backbone. And fourth, of the two dozen lipids and lipid derivatives used for mRNA delivery, about half utilize cyclic scaffolds (aryl, cyclohexyl, and piperazine) [2]. While unsuccessful with the aryl scaffolds, the opportunity to build off of cyclohexyl derivatives keeping with the metrics of symmetry and the use of a core backbone covalently linked to hydrophilic tendrils, as outlined above, exists. Be it cyclohexane 1,3,5-tricarboxylic acid or Kemp's triacid (*cis*,*cis*-1,3,5-trimethylcyclohexane-1,3,5-tricarboxylic acid), which offer the opportunity to study host–guest chemistry, the strategy of diversifying the carboxylic acid functionality using the Curtius rearrangement is viable, as documented herein, with the assembly of a carbamate derivative using, as the starting material, cyclohexane carboxylic acid.

4. Materials and Methods

All spectra were obtained as solutions in $CDCl_3$ having the following field strength: ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz). The NMR that generated the spectra was a JEOL ECA-500 spectrometer (JEOL Ltd., Tokyo, Japan) using JEOL Delta^{TM} Version 6.1.0 (MAC) software. Chemical shifts were reported in parts per million (ppm). Chemical shifts were referenced to δ 7.27 (^1H NMR) and δ 77.00 (^{13}C NMR). Infrared spectra were reported in wavenumbers (cm^{-1}) and recorded using a JASCO FT/IR-4100 (JASCO, Tokyo, Japan). For the synthetic procedures performed, all hazardous materials were handled while wearing protective gloves, protective clothing, eye protection, and face protection, and conducted in the hood. Additional considerations consisted of the following: TLC analyses were performed on flexible aluminum backed TLC plates with a fluorescent indicator. Detection was conducted by UV absorption (254 nm) followed by charring with 10% $KMnO_4$ in water. Solutions were concentrated in vacuo using a rotary evaporator, and the residue was purified by column chromatography (silica gel column (70–230 mesh, 60 Å)) followed by recrystallization (ethyl acetate and hexanes (1:1)). The HRMS data were generated at the University of South Alabama Mass Spectrometry Core Facility. The chemicals used for the synthetic procedure (cyclohexane carboxylic acid, diphenylphosphoryl azide, triethylamine, acetonitrile, ethyl acetate, hexanes) were reagent-grade or better. While DPPA is both commercially available and avoids the need to work with the far more labile acyl azide, the phophoryl azide is hazardous and should be handled, as would be the case with all hazardous materials, while wearing protective gloves, protective clothing, eye protection, and face protection. The Schiff base 2-((diphenylmethylene)amino)ethanol was prepared upon condensation benzophenone with 2-aminoethanol and used as is [11].

5. Experimental Process

*2-((Diphenylmethylene)amino)ethyl N-(Cyclohexyl)carbamate (**1**)*

Cyclohexane carboxylic acid (562 mg, 4.4 mmol, 1.0 equiv.) was added to a 50 mL round-bottomed flask (RBF) equipped with a magnetic stir bar and water-jacketed condenser. The carboxylic acid was then dissolved in acetonitrile (15 mL). After adding triethylamine (0.62 mL, 1.0 equiv.) and diphenylphosphoryl azide (1.2 g, 1.0 equiv.) at room temperature, the Schiff base 2-((diphenylmethylene)amino)ethanol (1.0 g, 1.0 equiv.) was added in one portion to the reaction mixture. After placing the setup under a blanket of argon, the reaction was allowed to warm to an external temperature of 80 °C and stirred overnight. Upon cooling to room temperature, the reaction mixture was concentrated in vacuo and chromatographed (TLC; SiO_2, 1:4 EtOAc:hexanes, R_f = 0.28). The column chromatography (SiO_2) gradient system increased in polarity using the following EtOAc/hexanes mixture, all representing three column equivalents: 1:8, 1:4, 1:2. The residue was then recrystallized (1:1 EtOAc/hexanes) resulting in 76 mg (5% yield) of a white, needle-like crystalline solid (mp 126.5–127 °C). Access to all the spectra acquired can be found within the Supplementary Materials.

^1H NMR (500 MHz, $CDCl_3$); δ 7.62–7.60 (m, 2H), 7.48–7.31 (m, 6H), 7.17–7.15 (m 2H), 4.58 (br s, 1H, CH(cyclohexyl)), 4.34 (t, 2H, *J* = 5.0 Hz), 3.58 (t, 2H, *J* = 5.0 Hz), 3.46 (br s, NH), 1.92–1.90 (m, 2H), 1.70–1.57 (m, 3H + H_2O), 1.35–1.26 (m, 2H), 1.18–1.07 (m, 3H). ^{13}C NMR ($CDCl_3$); δ 169.9, 155.7, 130.1, 128.6, 128.5, 128.5, 128.0, 127.8, 64.8, 53.0, 49.7, 33.4, 25.5, 24.8. IR (thin film); 3326, 3056, 2931, 2854, 1699, 1531 cm^{-1}. HRMS (LC-MS/MS) *m*/*z*: $[M + H]^+$ calcd for $C_{22}H_{26}N_2O_2$ 351.2066; found 351.2062.

6. Conclusions

As molecular structure impacts function, our focus on the development of effective tools of encapsulation and delivery of therapeutic molecules has shifted toward the install-

ment of carbamate functionality. Pairing this carboxylic acid derivative with existing alkyl chain motifs raises the biodegradability of the drug carrier. Furthermore, as the number of synthetic steps needed to install this functionality as part of the core backbone is half that currently used, the novel approach using the Curtius rearrangement as the key step streamlines the assembly of these next-generation lipid-like nanoparticles.

Supplementary Materials: The following are available online: ^{1}H NMR, ^{13}C NMR, HMQC, HETCOR, IR, and HRMS data of 2-((diphenylmethylene)amino)ethyl *N*-(cyclohexyl)carbamate (**1**).

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Conflicts of Interest: The authors declare no conflicts of interest.

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