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One Reaction Served Three Ways: The On-DNA Ugi 4C-3C Reaction for the Formation of Lactams

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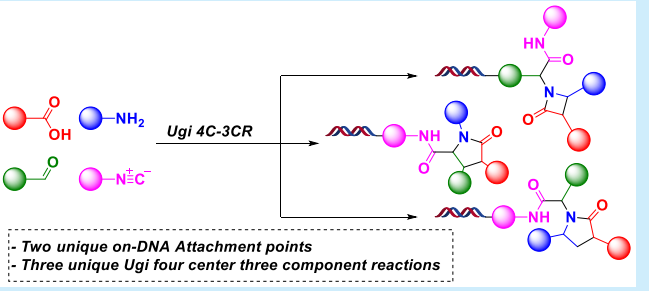
ABSTRACT: Developing new on-DNA reactions is paramount to the development of new encoded libraries in the pursuit of novel pharmaceutical lead compounds. Lactam-containing molecules have been shown to be effective in a wide range of therapeutic areas and therefore represent a promising target for further investigation by DNA-encoded library screening. In pursuit of this motif, we report a novel method for the introduction of lactam-containing structures onto a DNA headpiece through the Ugi four-center three-component reaction (4C-3CR). This novel method is successful in three different approaches to give unique on-DNA lactam structures: on-DNA aldehyde coupled with isonitriles and amino acids; on-DNA isonitrile coupled with aldehydes and amino acids; and on-DNA isonitrile coupled with amines and acid aldehydes.



DNA-encoded libraries (DELs) have matured as a platform for pharmaceutical discovery and have been instrumental in the discovery of new pharmaceutical lead compounds. Originally conceived as a concept by Brenner and Lerner in 1992,[1,2] DELs are collections of small molecules in which each unique compound is covalently bound to a unique oligonucleotide sequence, often termed the "DNA barcode". Each DNA strand is used to encode the unique building block used in each stage of the target small-molecule synthesis, which after screening against protein targets can be sequenced to map the specific building blocks used to compose the protein-binding compound. These concepts have now been well-explored throughout the pharmaceutical industry and academia.[3−5]

Further expansion of the DEL platform requires the development of new chemical processes to install small molecules onto the DNA headpiece. Chemistry in these systems is limited by the natural constraints of the DNA barcode: solution-phase reactions must be both robust enough to function in the partially aqueous conditions required to solubilize the oligonucleotide chain and target-specific enough to avoid reaction with the DNA.[6] Due to these limitations, early DELs were highly constrained by a small chemical toolbox. More recently, however, development of new chemistries has led to an explosion of newly possible on-DNA reactions. In this work, we describe the development of an on-DNA Ugi four-center three-component reaction (4C-3CR) for the formation of lactam-containing compounds.

With the discovery of penicillin in 1928,[7] lactams have represented a desirable motif in both modern antibiotics and other classes of small-molecule pharmaceuticals. The anti-cholesteremic drug ezetimibe (Zetia) represents a modern usage of the β-lactam structure.[8,9] γ-Lactam-containing active pharmaceutical compounds have also been discovered, such as the antiepileptic levetiracetam (Keppra) and the respiratory stimulant doxapram (Dopram).[10] Incorporation of this motif onto DNA can allow for the discovery of novel lactam-containing pharmaceutical lead compounds through screening of potential lactam-focused libraries.

One reported method for the formation of substituted lactam products is the Ugi 4C-3CR. The Ugi reaction is a multicomponent reaction between a carboxylic acid, an amine, an aldehyde, and an isonitrile (Figure 1).[11] A variation of the reaction is the Ugi 4C-3CR, which binds two of the centers to one component of the reaction for the formation of heterocycles (Figure 1).[12] This variation of the Ugi reaction has been reported in fully aqueous conditions, making it a promising candidate for translation to solution-phase on-DNA conditions.[13]

The Ugi reaction on DNA was first reported by the Brunschweiger group, who reported the synthesis of small libraries on solid-supported DNA strands.[14,15] Use of these solid supports allowed for application of fully organic conditions and provided greater stability in the face of highly

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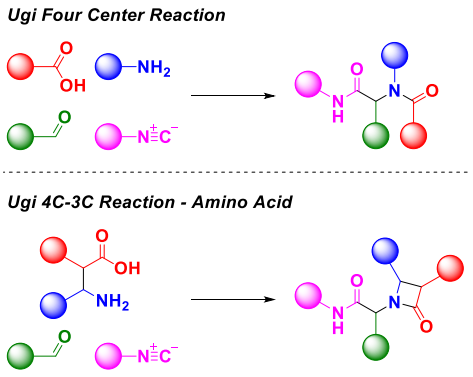
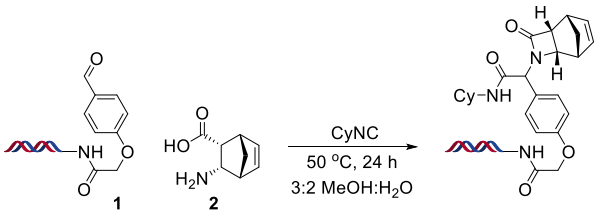


Figure 1. Ugi linear and 4C-3C reactions and products.

Table 1. Optimization of Ugi Teaction with On-DNA Aldehyde[a]



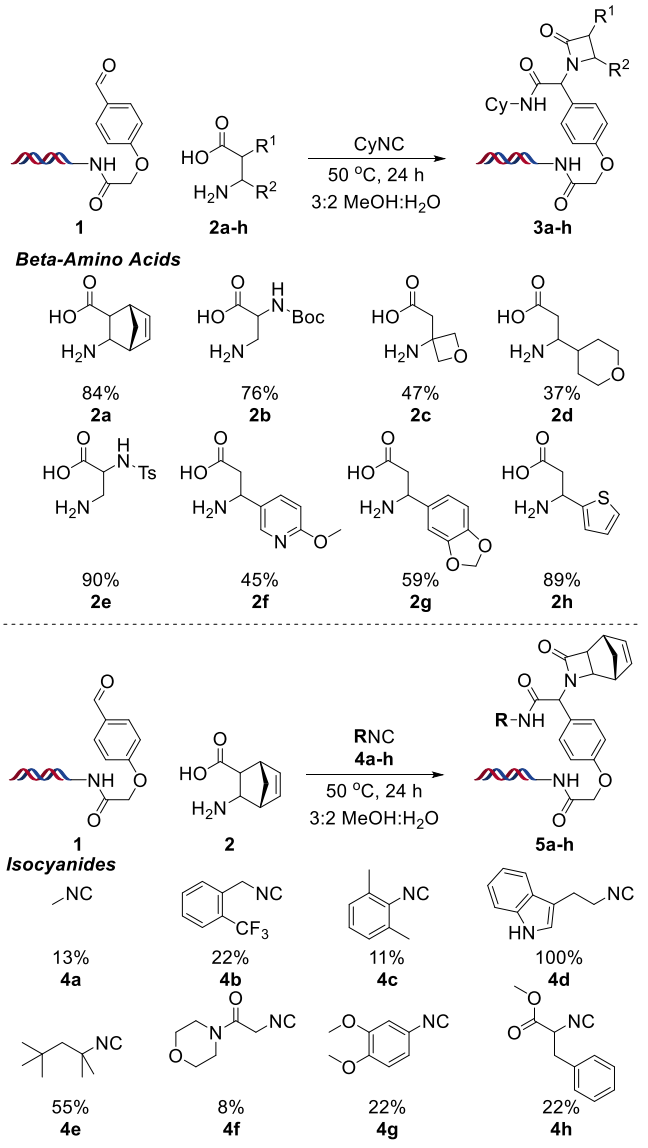
entry	solvent for **2**	solvent for CyNC [conc. (mM)]	equiv of CyNC	yield (%)
1	H_2O	H_2O [200]	100	5
2	MeOH	MeOH [200]	100	23
3[b]	MeOH	MeOH [200]	100	15
4	MeOH	MeOH [200]	1000	56
5	MeOH	MeOH [500]	1000	94
6[c]	MeOH	MeOH [500]	1000	36
7	H_2O	MeOH [500]	1000	84

[a]The product is presumed to be a mixture of diastereomers.[17] Conditions: a mixture of **1** in water (2 mM, 10 nmol), **2** in the indicated solvent (200 mM, 100 equiv), and CyNC (as indicated) was heated for 24 h at 50 °C. [b]Oligo was added as a 1 mM solution in 250 mM pH 9.4 sodium borate buffer. [c]The reaction was conducted at room temperature.

reactive isonitrile components. These conditions, however, cannot be directly translated to solution-phase reactions, as the use of fully organic conditions renders the oligo insoluble. A linear form of the Ugi reaction has also been studied by the Obika group in solution phase.[16] This linear Ugi reaction, however, was only successful when telescoped: the Ugi reaction was completed in organic conditions off-DNA, and then the DNA headpiece was coupled to the Ugi product in the same pot. Additionally, these solution-phase reactions were not used in the direct synthesis of heterocycles but were used only in the construction of linear compounds.

In our hands, initial reactions on DNA with headpiece **1**, amino acid **2**, and cyclohexylisonitrile (CyNC) conducted in fully aqueous conditions produced only trace quantities of Ugi product (Table 1, entry 1). Addition of **2** and CyNC in methanol, however, drove the reaction to produce greater quantities of β-lactam product (Table 1, entry 2). While addition of **2** in methanol led to higher yields, **2** was largely insoluble in methanol and was added as a slurry. Addition of buffer hindered formation of the product compared to the unbuffered example (Table 1, entry 3), a trend which proved true for buffers at basic, neutral, and acidic pH. Increasing the

Scheme 1. Substrate Scope of On-DNA Aldehyde with Amino Acids and Isonitriles[a]



[a]All products are presumed to be mixtures of diastereomers.[17] Conditions: a mixture of **1** in water (2 mM, 10 nmol), **2** in H_2O (200 mM, 100 equiv), and CyNC in MeOH (500 mM, 1000 equiv) was heated for 24 h at 50 °C.

number of equivalents of isonitrile relative to amino acid while retaining the concentration of CyNC solution led to a moderately increased yield (Table 1, entry 4). We hypothesize that this increase in yield relative to an increased portion of MeOH in the solvent mixture is due to the known instability of isonitriles toward hydrolysis in the presence of aqueous acid.[18] Increasing the concentration of CyNC solution while retaining the higher number of equivalents proved successful in increasing the yield to 94% (Table 1, entry 5). Heating was found to be necessary to achieve high yields of the desired product (Table 1, entry 6). Addition of **2** in H_2O alongside CyNC in MeOH led to a small decrease in yield (Table 1, entry 7) but was more successful in solubilizing the amino acid, which proved necessary for the use of other amino acids.

A small scope of β-amino acids was evaluated for the Ugi reaction with **1** and CyNC. The optimized conditions from

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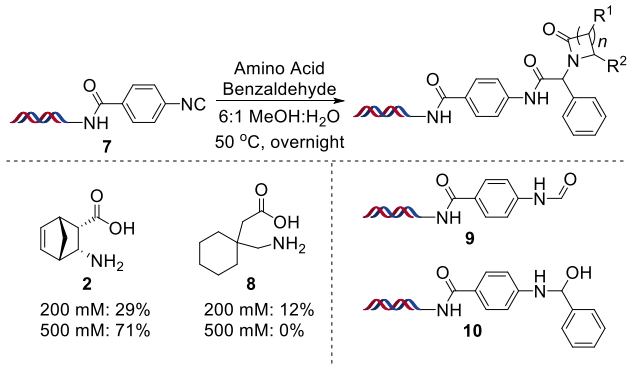
Scheme 2. Initial Reactions with 7 and Observed Side Products[a]



[a]All products are presumed to be mixtures of diastereomers.[17] Conditions: a mixture of **7** in water (2 mM, 10 nmol), **2** in MeOH (200 mM at 100 equiv, 500 mM at 1000 equiv), and benzaldehyde in MeOH (500 mM, 1000 equiv) was heated overnight at 50 °C.

Table 2. Optimization of the On-DNA Isonitrile Ugi Reaction[a]

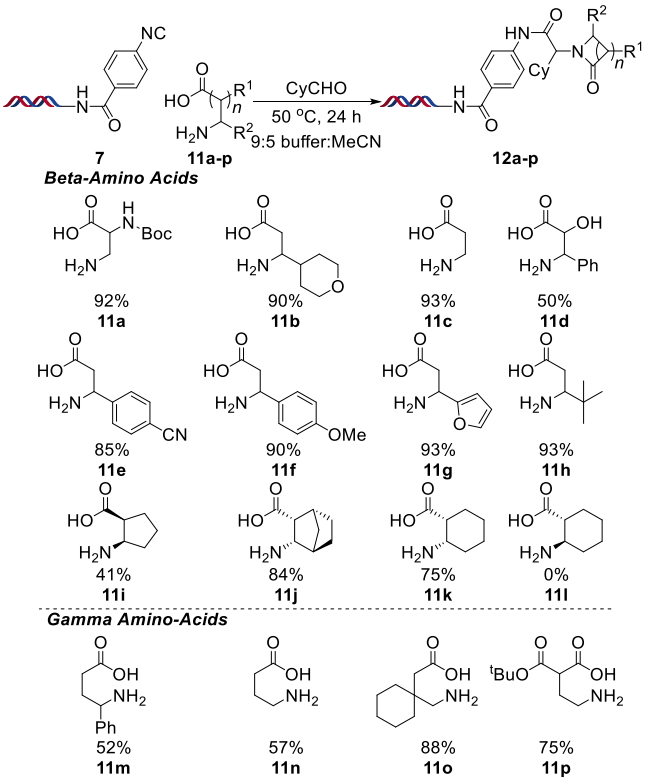
entry	buffer pH	RCHO	solvent for RCHO	conc. of **8** (mM)	yield (%)
1	8.0	PhCHO	MeOH	200	0
2	9.4	PhCHO	MeOH	200	0
3	7.0	PhCHO	MeOH	200	16
4[b]	7.0	CyCHO	MeOH	200	53
5[c]	**7.0**	**CyCHO**	**MeCN**	**125**	**94**

[a]The product is presumed to be a mixture of diastereomers.[17] Conditions: a mixture of **7** in water (2 mM, 10 nmol), **8** in 1:1 MeOH/buffer (X mM, 100 equiv), and RCHO in the indicated solvent (500 mM, 1000 equiv) was heated for 24 h at 50 °C. [b]**7** was added in 500 mM pH 7.0 sodium borate buffer. [c]**7** and **8** (500 equiv) were added in 500 mM pH 7.0 sodium borate buffer.

Table 1, entry 7 were used, as many amino acids tested were found to be insoluble in MeOH. Both alkyl and aryl substituents were tolerated, as well as substituents at both the α and β positions of the amino acid (Scheme 1, **2b–f**). Amino acids containing electron-rich ring systems and heterocycles also produced moderate to high yields of product (Scheme 1, **2g** and **2h**). Extension of this system to the formation of γ-lactams was less successful, providing only low yields of the desired products (0–8% yield; see Scheme S2). While screening isonitriles in the Ugi reaction, only two of the isonitriles produced yields over 50% (Scheme 1, **4d** and **4e**), with the rest producing less than 25% yield of the desired Ugi product. These reactions generally contained only starting material and product by LCMS analysis, implying that there was no significant side product formation on DNA.

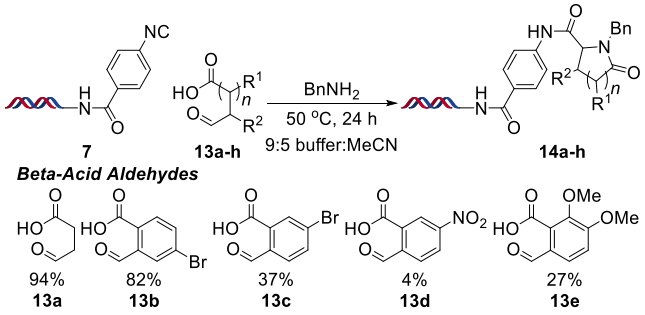
Development of on-DNA reactions in pursuit of new library synthesis relies on large numbers of potential building blocks to build diversity. As variation of the isonitrile in the above reaction proved limiting, a new scheme was devised that placed the isonitrile on DNA. This design presented a unique challenge, as the need for an appropriate chemical handle for

Scheme 3. Substrate Scope of On-DNA Isonitrile with Amino Acids[a]



[a]All products are presumed to be mixtures of diastereomers.[17] Conditions: to **7** in 500 mM pH 7.0 sodium borate buffer (2 mM, 10 nmol) were added MeCN (5 μL), **11** in 500 mM pH 7.0 sodium borate buffer (200 mM, 500 equiv), and CyCHO in MeCN (500 mM, 1000 equiv), and the mixture was heated for 24 h at 50 °C.

Scheme 4. Substrate Scope of On-DNA Isonitrile with Acid Aldehydes[a]



[a]All products are presumed to be mixtures of diastereomers.[17] Conditions: to **7** in 500 mM pH 7.0 sodium borate buffer (2 mM, 10 nmol) were added MeCN (5 μL), **13** in 500 mM pH 7.0 sodium borate buffer (200 mM, 500 equiv), and $BnNH_2$ in MeCN (500 mM, 1000 equiv), and the mixture was heated for 24 h at 50 °C.

installation onto the headpiece had to be balanced with the susceptibility of the isonitrile to hydrolysis. Headpiece **7** was devised to balance the relative lower nucleophilicity of an aryl isonitrile[19] with the need for an acid building block to complete the common HATU coupling.[6]

Reactions were designed to utilize this new headpiece **7** with free aldehydes and β- or γ-amino acids. Initial reactions with **2** were successful in producing β-lactam products at high

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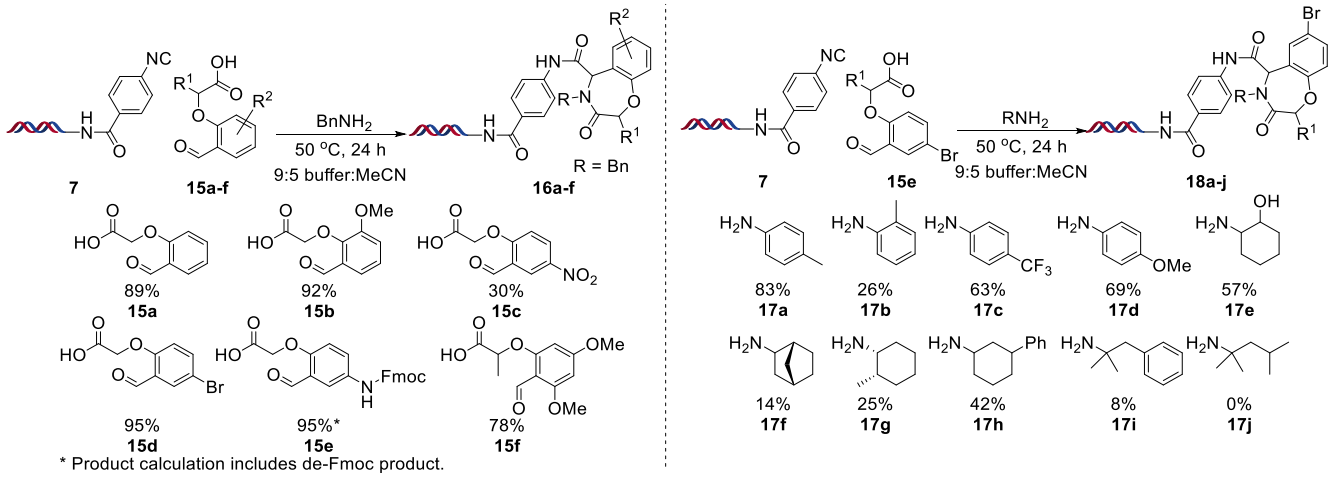
Scheme 5. Substrate Scope of On-DNA Isonitrile with Acid Aldehydes and Amines to Form Dihydrobenzoxazepinones[a]



[a]All products are presumed to be mixtures of diastereomers.[17] Conditions: to **7** in 500 mM pH 7.0 sodium borate buffer (2 mM, 10 nmol) were added MeCN (5 μL), **15** in 500 mM pH 7.0 sodium borate buffer (200 mM, 500 equiv), and RNH_2 (**17**) in MeCN (500 mM, 1000 equiv), and the mixture was heated for 24 h at 50 °C.

concentrations of amino acid (Scheme 2). However, reactions to form γ-lactam products proved more challenging, leading to low yields when amino acid **8** was used at both low and high concentrations. The major byproduct of these reactions was the hydrolysis product **9**, which is formed from **7** in the presence of acid.[20] Indeed, investigation of the resultant solution found a pH of <5, indicating that a significant concentration of acid was present in the course of the reaction.

Adjusting the pH of the reaction using basic buffer (500 mM sodium borate, both pH 8.0 and pH 9.4) was found to only produce aldehyde adduct **10** with no detected Ugi product (Table 2, entries 1 and 2). Holding the reaction pH at 7.0, however, successfully produced the desired γ-lactam product from amino acid **8** in 16% yield (Table 2, entry 3). Changing the aldehyde from aryl benzaldehyde to aliphatic cyclohexylcarbaldehyde increased the yield of the desired γ-lactam Ugi product to 53% (Table 2, entry 4). Further optimization was achieved by addition of the amino acid as a 125 mM solution in pH 7.0 buffer and the aldehyde as a 500 mM solution in acetonitrile rather than methanol, leading to 94% yield (Table 2, entry 5).

This reaction was found to be widely applicable with both β- and γ-amino acids. When β-amino acids are employed, the reaction tolerates α-substituted, β-substituted, and α,β-disubstituted amino acids (Scheme 3, **11a**, **11b**, and **11d**) Amino acids with electron-poor or electron-rich arenes as well as aromatic heterocycles are tolerated (Scheme 3, **11e–g**). Highly hindered amines are tolerated (Scheme 3, **11h**). *Cis* cyclic systems are tolerated (Scheme 3, **11i–k**), but not those in a *trans* conformation (Scheme 3, **11l**). γ-Amino acids, while successful in yielding the desired Ugi product, were generally lower yielding (Scheme 3, **11m–p**).

As an alternative method, the components of the reaction were shuffled to utilize an on-DNA isonitrile, an acid aldehyde, and a free amine. The conditions used in the above on-DNA isonitrile reaction were applicable to the new reaction without further optimization. γ-Lactams were successfully synthesized in high yields. Both flexible C(sp^3) (Scheme 4, **13a**) and rigid C(sp^2) (Scheme 4, **13b–e**) acid components were successful in producing γ-lactam products. Substituents *para* to the acid component were tolerated (Scheme 4, **13b**), while similar substituents *meta* to the aldehyde component were lower-yielding (Scheme 4, **13c–e**). The reaction with acid aldehydes was able to be extended in lower yields to the formation of δ-lactams and ε-lactams (Scheme S5).

Dihydrobenzoxazepinone rings were also successfully synthesized on DNA using acid aldehyde starting materials. Electron-donating substituents are well-tolerated (Scheme 5, **15b**), while very strong electron-withdrawing substituents produce lower yields of Ugi product (Scheme 5, **15c**). This reaction also tolerates bulky groups on the ring (Scheme 5, **15e**) and on the alkyl linker (Scheme 5, **15f**). Amines were also screened in the reaction with **15e**, and moderate to high yields were observed for aryl and cyclohexyl amines. Aromatic amines containing *para* substituents (Scheme 5, **17a**) produced higher yields of Ugi product than similar *ortho*-substituted amines (Scheme 5, **17b**). Both electron-rich and electron-poor substituents were tolerated similarly (Scheme 5, **17c** and **17d**). Amines bound to a cyclohexyl ring produced only moderate yields (Scheme 5, **17e–h**), while tertiary, sterically hindered amines produced only low yields of Ugi product (Scheme 5, **17i** and **17j**).

In summary, we have described the development of several solution-phase on-DNA Ugi 4C-3CRs for the formation of lactams and dihydrobenzoxazepinones. High yields were achieved using both an on-DNA aldehyde handle and an on-DNA isonitrile. Both on-DNA reactive handles were used in the formation of β- and γ-lactams with a variety of amino acids and acid aldehydes. The wide range of commercially available amino acids and acid aldehydes allow these reactions to be exciting options for the formation of diverse DNA-encoded libraries based on four-, five-, six-, and seven-membered lactams.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its online Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.2c04043.

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DNA headpiece structure, supporting figures, general procedures and reagents used, and copies of LCMS and deconvoluted spectra for all on-DNA reactions (PDF)

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Notes

The authors declare no competing financial interest.

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