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ARTICLE

Exploration of a Au(I)-mediated three-component reaction for the synthesis of DNA-tagged highly substituted spiroheterocycles

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We demonstrate a Au(I)-mediated three-component reaction to DNA-tagged highly substituted 6-oxa-1,2-diazaspiro[4.4]nonanes from either DNA-coupled aldehydes, hydrazides, or alkynols. The choice of the starting material coupled to the DNA tag was critial for the purity of the product as the DNA-aldehyde conjugate yielded the purest products, whereas the alkynol- and hydrazide conjugates returned complex product mixtures. The reaction was compatible with thymine-, cytosine-, and, surprisingly, with adenine-DNA, while guanine-containing DNA strands were degraded under the reaction conditions.

Introduction

Genetically tagged collections of drug-like compounds (Fig. 1A), called DNA-encoded libraries (DELs), have emerged as a valuable addition to the arsenal of technologies for the identification of small organic molecules binding to a target protein.[1] In contrast to discrete screening libraries of small molecules, DELs are much more efficiently handled as large compound mixtures. These are screened by an efficient, generic assay based on the principle of selection. The whole encoded library is incubated as a mixture with a target protein immobilized on a surface, washing steps enrich compounds binding to the protein. Protein binders are then eluted, and detected by sequencing of their DNA tag. DELs have yielded many bioactive compounds, some of them are useful chemical biology probes, and a few attained status as drug candidates.[1] Several encoding strategies have been developed by academic, and industrial research groups for drug discovery.[2] Commonly, encoded libraries are synthesized by combinatorial cycles of alternated synthesis, and encoding steps tracking the synthesis. Synthesis methods employed for the synthesis of these libraries must give high yields, display a broad scope, tolerate water as (co)-solvent, and must preserve the genetic information.[3] Workhorse reactions meeting all these requirements are e.g. carbonyl reactions, nucleophilic substitution of reactive halides, and C-C-cross coupling reactions. All of these reactions couple building blocks, yielding in effect rather planar and stretched out structures.[4] Accessing DNA-tagged three-dimensional chemical matter from simple, and readily available starting materials to provide DELs with shape diversity is currently a

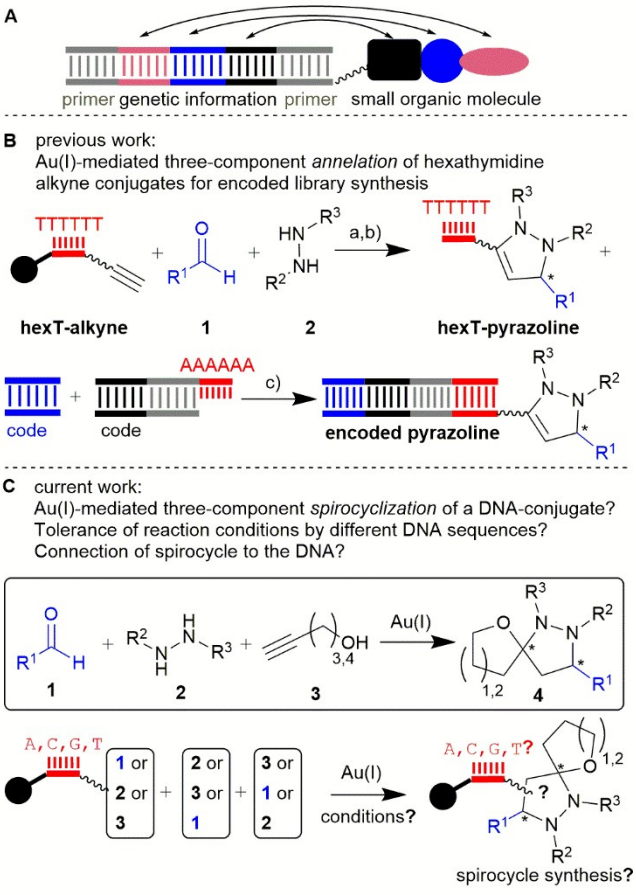

Fig. 1 Previous and current work towards expanding the chemical space of genetically tagged libraries. A) Generic structure of a DNA-encoded compound. B) The TiDEC approach to a DNA-encoded library of pyrazolines. C) Investigations in the suitability of a Au(I)-catalyzed spirocycle synthesis for encoded library synthesis.

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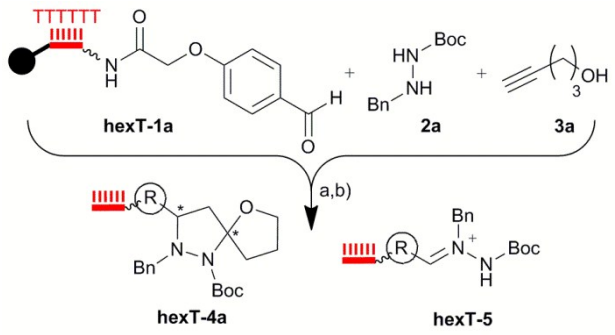
major challenge in the field. Diverse (partially) saturated scaffolds are attractive library constituents as they project their substituents into space.[5]

Table 1 Reaction optimization for the conversion of **hexT-1a** to spirocycle **hexT-4a** by Au(I)-mediated three-component reaction. a) Conditions see table, eq. relative to the DNA, room temperature, tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride, $AgSbF_6$;[a] b) aq. NH_3/$MeNH_2$. R: *para*-(aminocarbonylmethyleneoxy)phenyl. Wavy bond to hexT: 5'-(C6)-amino-linker, bold bond: connection to solid phase, filled circle: solid phase.



entry	solvent	cat. (eq.)	**2a** (eq.)	**3a** (eq.)	time [h]	**4a** [%][b]	**5** [%][b]
1[c]	THF	10	100	200	20	-	< 5
2[c]	THF	10	500	1000	20	-	< 5
3[c]	THF	50	500	1000	20	40	< 5
4[c]	THF	100	500	1000	20	50	< 5
5[c]	THF	250	500	1000	14	55	35
6[c]	THF	250	500	1000	18	75	20
7[c]	THF	250	500	1000	20	90	< 5
8	THF	250	-	-	20	-	-
9	THF	-	500	1000	20	-	-
11	MeOH	250	500	1000	14	-	90
12	MeCN	250	500	1000	14	45	45
13	DMF	250	500	1000	14	-	75
14	$C_2H_4Cl_2$	250	500	1000	14	10	55
15	CH_2Cl_2	250	500	1000	14	10	70
16	toluene	250	500	1000	14	15	50

[a] Reaction performed on a 30 nmol scale; [b] conversion estimated by HPLC analysis of the crude product; [c] note that these conjugates contain a PEG(4) linker.

They can be incorporated into DELs through functionalized scaffolds,[6] or by development of DNA-compatible synthesis methods. Examples for the latter are the Diels-Alder reaction, a tertiary amino effect reaction to spirocycles, a few heterocycle-forming reactions, and the metathesis reaction, reflecting the strong interest in expanding DEL synthesis methods.[7] Metal-catalysis is a vibrant research field in organic chemistry, enabling synthesis of ever more diverse structures from simple starting materials.[8] However, many metal ion catalysts, displaying Lewis acid and oxidizing properties, are interacting or even reacting with purine nucleosides in a DNA strand, causing eventually depurination of DNA.[9] Thus, they usually demand the chemically much more stable PNA for tagging.[10] We recently described an encoding strategy called TiDEC (oligothymidine initiated DNA-encoded chemistry), which initiates DEL synthesis with a chemically surprisingly stable hexathymidine adapter "hexT".[11] The hexT adapter oligonucleotide tolerated the harsh reaction conditions of Au(I) catalysis giving access to highly substituted pyrazol(in)es from simple starting materials by a Au(I)-mediated annulation reaction for DEL-synthesis (Fig. 1B).

Spirocycles are archetypes of three-dimensional structures, and well represented in natural products, yet they are compared to more simple (hetero)cyclic structures much less intensely explored in drug research.[12] Here, we systematically investigated a Au(I)-mediated three-component reaction to hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates **4** from readily available aldehydes **1**, hydrazides **2**, and alkynols **3**.[13] Additionally, the connectivity between hexT and spirocycle, and the tolerance of different DNA sequences to optimized conditions for the Au(I)-mediated reaction were explored (Fig. 1C).

Results and discussion

The synthesis of the hexT-diazaoxaspironane conjugate **hexT-4a** was explored with the controlled pore glass (CPG) solid support-bound hexT-aldehyde conjugate **1a**, the non-symmetrically substituted hydrazide **2a**, and the alkynol **3a** using tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride and $AgSbF_6$ as catalyst (Table 1). Following reaction, the DNA conjugates were cleaved from the solid phase with a mixture of aqueous ammonia/methylamine, HPLC-purified, and analyzed by HPLC and MALDI MS.

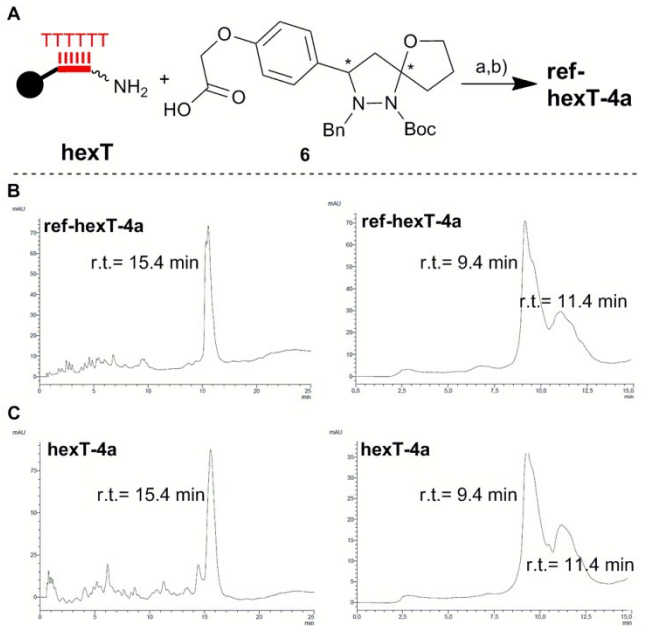


Fig. 2 Synthesis and comparison of the reference **ref-hexT4a** with **hexT4a** (Table 1). A) Synthesis of the control **ref-hexT-4a**; a) HATU, DIPEA, DMF, room temperature, 4 hours; b) aq. NH_3/$MeNH_2$. B) HPLC analysis of **ref-hexT-4a**. C) HPLC analysis of **hexT-4a**. Wavy bond to hexT: 5'-(C6)-amino-linker, bold bond: connection to solid phase, filled circle: solid phase, r.t.: retention time.

In a first series of reactions run in THF at room temperature for 20 hours, equivalents of reactants and catalyst were explored (Table 1, entries 1-4, and 7, see also Table S1, and ESI for extended information, and HPLC traces of all crude reactions). Increasing the excess of hydrazide **2a** to 500 equivalents,

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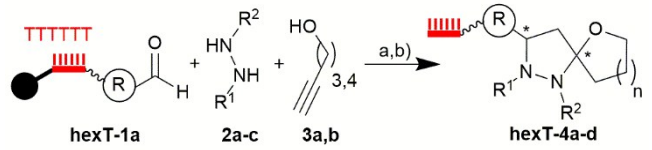
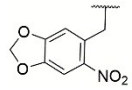
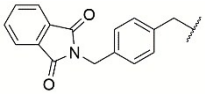
alkynol **3a** to 1000 equivalents, and catalyst to 250 equivalents were in our hands critical for product yield. Shorter reaction times (entries 5,6) returned a second product with the mass of the plausible azomethine imine intermediate **hexT-5** which we found stable during cleavage of DNA with concentrated aqueous ammonia and HPLC purification (ESI, Fig. S8). Among the solvents that were investigated (entries 11-16), the polar aprotic MeCN gave similar results to THF, whereas the reaction yielded predominantly, if not exclusively the azomethine imine intermediate **hexT-5** in all non-polar solvents, in the polar aprotic solvent DMF, and also in the polar protic MeOH. These results are in agreement with previous observations.[13] Control reactions either without catalyst or without reactants returned the starting material peaks in the HPLC analysis as expected (entries 8-9).

Table 2 Scope of the reaction yielding hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugates **hexT-4**. a) Reaction condition No. 7 (Table 1); b) AMA (aqueous ammonia (30 %)/ aqueous methylamine (40 %), 1:1, vol/vol), 30 min, room temperature). R: *para*-(aminocarbonylmethyleneoxy)phenyl. Filled circle denotes solid support (controlled pore glass, CPG); wavy bond to hexT: 5'-(C6)-amino-linker.



entry	hexT	2		3	n	*mass calc.* mass found[a]
		R^1	R^2			
1	4a		*t*-Boc	a	1	*2392.7* 2394.5
2	4b		*t*-Boc	a	1	*2302.6*[b] 2305.1[b]
3	4c		Ac	a	1	*2363.7* 2366.2[c]
4	4d		*t*-Boc	b	2	*2406.8* 2410.3

[a] measured by MALDI-MS; [b] loss of the photolabile 3,4-methylendioxy-6-nitrobenzyl group upon irradiation in the mass spectrometer; [c] phthalimide protective group removed in the product.

In order to confirm formation of the spirocycle conjugate **hexT-4a**, we synthesized and fully characterized a reference spirocycle **6** that was then coupled by amide formation to 5'-amino-linker-modified hexT yielding the reference compound **ref-hexT-4a** (Fig. 2A, and ESI Fig S12). Both compounds **hexT-4a** and **ref-hexT-4a** showed the same mass in the MALDI analysis, and more importantly, the HPLC trace of the reference hexT-spirocycle conjugate mirrored the product of the Au(I)-mediated spirocycle synthesis (Fig. 2B,C) lending credibility to the formation of the spirocycle conjugate **hexT-4a** suggested by reaction mechanism (Table 1), HPLC analysis, and matching mass spectrum. As the Au(I)-catalyzed reaction furnishes diastereomers, we were curious to learn whether performing the reaction in the presence of the solid support-bound DNA has any impact on the diastereomeric ratio of the products. Comparison of the HPLC traces of compounds **hexT-4a** and **ref-hexT-4a** suggests that the hexT DNA did not impact the diastereomeric ratio of the product (Fig. 2B,C).

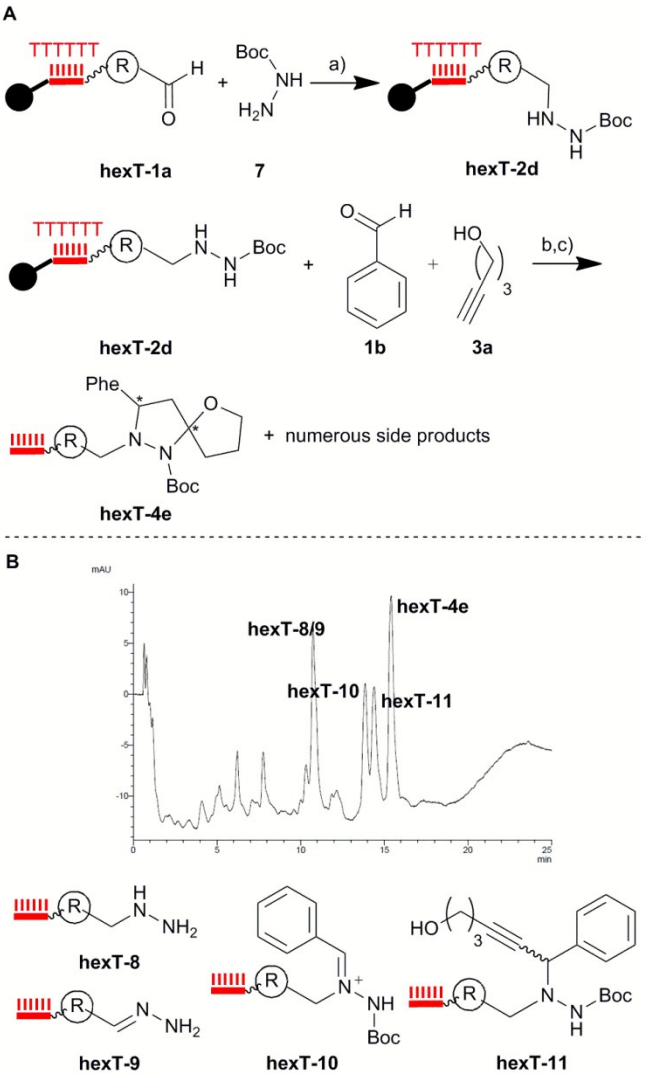


Fig. 3 Synthesis of the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **4e**. A) Reaction scheme of the spirocycle formation. The synthesis route starts from the solid support bound hexT-aldehyde **1a**, which is reacted with carbazate **7** to hexT-hydrazide **2d**, the hexT hydrazide conjugate is then reacted with benzaldehyde **1b** and pentynol **3a** to the spirocycle **4e**. Reagents and conditions: a) $NaBH_3CN$, aqueous 3-(*N*-morpholino)propanesulfonic acid buffer, room temperature, overnight; b) 250 eq. tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride, 250 eq. $AgSbF_6$, 1000 eq. **1b**, 1000 eq. **3a**, THF, room temperature, 20 hours; c) aq. NH_3/$MeNH_2$, room temperature, 30 min. B) HPLC trace of the crude reaction mixture showing formation of numerous products besides the target spirocycle **4e**. R: *para*-(aminocarbonylmethyleneoxy)phenyl. Wavy bond to hexT: 5'-(C6)-amino-linker, bold bond: connection to solid phase, filled circle: solid phase.

Next, the product scope of the reaction towards a projected library synthesis was explored: a hydrazide with the photocleavable 3,4-methylenedioxy-2-nitro-benzyl protective group **2b** that is removed upon UV-irradiation at 365 nm, a hydrazide that is substituted with an acetyl group instead of Boc, and a protected amino-substituted benzyl group **2c**, and also the hexinol **3b** all furnished the target hexT spirocycle

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conjugates **4b-d** (Table 2, Table S2, see ESI for MALDI MS spectra of all products).

Multi-component reactions open up the very attractive opportunity to exploit each reactant as connection to the DNA. Thus, library diversity can be efficiently accessed with one chemical transformation, and overlapping sets of reactants. Therefore, we next investigated the feasibility of conjugating the hydrazide to the hexT adapter oligonucleotide, and converting this compound to the target spirocycle by the Au(I)-mediated reaction.

Table 3 Reaction optimization for the conversion of the hexathymidine-hydrazide conjugate **hexT-2d** to spirocycle **hexT-4e** by Au(I)-mediated three-component reaction (see Fig. 3).[a]

entry	solvent	cat. (eq.)	**1b** (eq.)	**3a** (eq.)	time [h]	**4e** [%][b]
1	MeOH	250	500	1000	20	15
2	DMF	250	500	1000	20	35
3	MeCN	250	500	1000	20	25
4	THF	250	500	1000	14	20
5	THF	250	500	1000	20	40
6	1,2-DCE	250	500	1000	20	5

[a] reaction performed on a 30 nmol scale; [b] conversion estimated by HPLC analysis of the crude reaction.

This approach to the target spirocycle would be more attractive from a library synthesis perspective as it benefits from the abundant availability of aldehyde building blocks. We first synthesized the hexT-hydrazide conjugate **hexT-2d** from the aldehyde **hexT-1a** by reductive amination with *tert*-butyl carbazate (Figs. 3, and S3, ESI). This conjugate **hexT-2d** was then reacted with benzaldehyde **1b**, and pentynol **3a** to furnish hexT-spirocycle **4e** under several reaction conditions (Fig. 3A, Tables 3 and S3, ESI). Again, long reaction times, large excess of reactants and THF as solvent turned out to be required for product formation (Tables 3, and S3, ESI). However, conversion rates to the hexT-spirocycle **4e** were in our hands much lower than those to hexT-spirocycle **4a**, and in contrast to the latter, we obtained **4e** as part of a complex compound mixture in each reaction (Fig. 3B). This product mixture contained the co-eluting Boc-cleavage products **hexT-8**, and the imine **hexT-9** which resulted from incomplete reduction in the preceding step; the azomethine imine side product **hexT-10**, which formed in analogous manner to the side product **hexT-5** observed in the reaction to **hexT-4a**; a side-product that we tentatively assigned the structure of the Mannich-type product **hexT-11**, as a similar side-product formed in the synthesis of pyrazolines[11] (see also Fig. S19, ESI); and the target product **4e** which eluted as a distinct peak with nearly the same retention time as **hexT-4a** (Fig. S20, ESI). The side products were characterized with control experiments, and by MALDI analysis (see ESI). Next, the scope of the reaction was explored with a few aldehydes. Substituted electron-rich and electron-poor aromatic, a heterocyclic, and an aliphatic aldehyde all furnished the target hexT-conjugates **4f-i** (Table S4, ESI). However, we obtained in each reaction a compound mixture

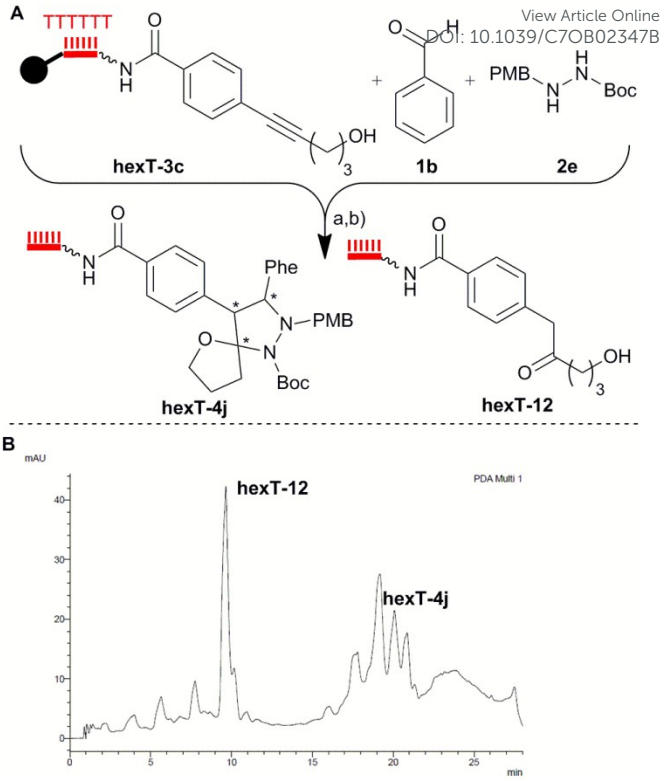


Fig. 4 Synthesis of the hexT-6-oxa-1,2-diazaspiro[4.4]nonane conjugate **4j** from hexT-alkynol **3c**. A) reaction scheme of the spirocycle formation, reagents and conditions: a) 30 nmol scale, 250 eq. tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride, 250 eq. $AgSbF_6$, 1000 eq. **1b**, 500 eq. **2e**, MeCN, 45°C, 17 hours; b) aq. $NH_3/MeNH_2$, room temperature, 30 min. B) HPLC trace of the crude reaction mixture showing formation of the water addition product **hexT-12** as major product, and the target spirocycle **4j** closely eluting with several side products. PMB: *para*-methoxybenzyl. R: *para*-(aminocarbonylmethyleneoxy)phenyl. Wavy bond to hexT: 5′-(C6)-amino-linker, bold bond: connection to solid phase, filled circle: solid phase.

that required HPLC purification, calling the utility of this approach for library synthesis in question.

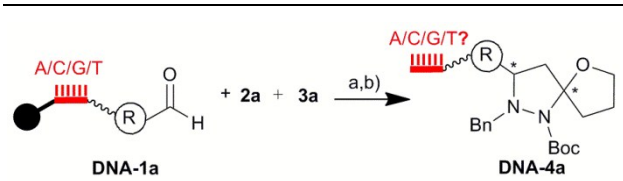
Table 4 Reaction optimization for the conversion of the hexythymidine-alkynol conjugate **hexT-3c** to spirocycle **hexT-4j** by Au(I)-mediated three-component reaction (see Fig. 4).[a]

entry	solvent	cat.	T [°C]	time [h]	add.[b]	**4j** [%][c]
1	MeCN	Au(I)/Ag(I)	45	17	-	-
2	MeCN	Au(I)/Ag(I)	25	17	MS 5Å	-
3	MeCN	Au(I)/Ag(I)	45	17	MS 5Å	40
4	MeCN	Au(I)/Ag(I)	50	17	MS 5Å	30
5	MeCN	Au(I)/Ag(I)	55	17	MS 5Å	25
6	MeCN	Au(I)	45	17	MS 5Å	-
7	MeCN	Ag(I)	45	17	MS 5Å	-
8	THF	Au(I)/Ag(I)	45	17	MS 5Å	30
9	THF	Au(I)/Ag(I)	45	41	MS 5Å	10
10	$C_2H_4Cl_2$	Au(I)/Ag(I)	45	17	MS 5Å	25
11	toluene	Au(I)/Ag(I)	45	17	MS 5Å	trace
12	DMF	Au(I)/Ag(I)	45	17	MS 5Å	-

[a] reaction performed on a 30 nmol scale with 1000 eq. of aldehyde **1b**, 500 eq. of hydrazide **2e**, and 250 eq. of tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride, $AgSbF_6$; [b] additive; [c] conversion estimated by HPLC analysis of the crude reaction.

To complete the walk around the three component reaction, we coupled an alkynol (**19**, Fig. S4, ESI) to the hexT furnishing **hexT-3c**, and reacted this hexT-conjugate with benzaldehyde **1b** and hydrazide **2e** to the hexT-spirocycle **4j** at elevated temperature of 45 °C (Fig. 4A).[13] Initial efforts to obtain the target molecule were thwarted by the formation of the water adduct **hexT-12** as sole product (Table 4, entry 1, see extended Table S5, ESI). The water adduct can be explained by the Au(I)-mediated formation of a highly reactive cyclic enol ether[13] from the alkynol **hexT-3c** which is hydrolysed by the massive amounts of water generated by the condensation of aldehyde **1b** and hydrazine **2b**. Therefore, all further reactions were performed adding the water scavengers $MgSO_4$ or molecular sieves to the reaction mixture. Under these conditions, formation of a product corresponding to the mass of the hexT-spirocycle conjugate **hexT-4j** was detected (entry 3). However, product yields were modest, and made an investigation of the reaction conditions necessary. Running the reaction at 25°C returned unreacted starting material, increasing the temperature to 50°C or 55°C (entries 2-5), and also prolonged reaction time at 45°C resulted in lower product formation due degradation (entries 9). Among the solvents that were tested, acetonitrile, THF, and dichloroethane yielded the spiroheterocycle (entries 3, 8, 10), whereas no or only traces of the product were detected in toluene and DMF. Thus, the spirocycle formation displayed a narrow window with respect to reaction conditions, and it required the extra effort to keep the solvent dry in order to reduce formation of the water adduct **hexT-12**.

Table 4 Tolerance of different DNA sequences to the Au(I)-mediated three-component reaction. Reagents and conditions: a) 30 nmol scale, 250 eq. tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride, 250 eq. $AgSbF_6$, 500 eq. **2a**, 1000 eq. **3a**, THF, room temperature, 20 hours; b) aq. $NH_3/MeNH_2$. R: *para*-(aminocarbonylmethyleneoxy)phenyl. Wavy bond to DNA: 5'-(C6)-amino-linker, bold bond: connection to solid phase, filled circle: solid phase.



entry	sequence 5'-3'	DNA-4a mass calc.	DNA-4a mass found[a]
1	**4a**-TTTTTT	2392.8	2394.5
2	**4a**-CCCCCC	2302.7	2304.7
3	**4a**-TCTCTC	2347.7	2351.9
4	**4a**-AAAAAA	2446.9	2448.7
5	**4a**-TCATCT	2371.7	2374.2
6	**4a**-GGGGGG	2542.8	-
7	**4a**-CAGTCG	2421.7	-

[a] measured by MALDI-MS.

The reaction gave the target products with benzaldehydes, and cyclopropyl carboxaldehyde, and with several unsymmetrically substituted hydrazides. The latter could be substituted with different benzyl groups, and with a *tert*-Boc or an acetyl group (Table S6, ESI). However, we obtained under all tested conditions complex product mixtures from which the target spirocycles had to be isolated by careful ion-pair HPLC purification, and we were not able to completely suppress the addition of water to the starting material **hexT-3c**. Thus, starting library synthesis from the hexT-alkynol conjugate **hexT-3c** is according to these results not a viable option.

Next, we were curious to learn about the tolerance of different DNA sequences to the optimized reaction conditions established for the Au(I)-mediated synthesis of hexT-diazaoxaspirononane **4a**. For this experiment aldehyde conjugates of six different solid phase-bound DNA sequences were synthesized: **hexC-1a**, **hexTC-1a**, **hexA-1a**, **hexG-1a**, **hexACT-1a**, and **hexACGT-1a**. These were reacted with each 500 eq. of hydrazide **2a** and 1000 eq. of pentynol **3a**, and 250 eq. of Au(I) and $AgSbF_6$ to furnish DNA-6-oxa-1,2-diazaspiro[4.4]nonane conjugates. In addition to the hexT-conjugate **hexT-4a**, we were able to isolate distinct product peaks for the oligopyrimidine conjugates **hexC-4a** and **hexTC-4a**. The nucleosidic bond of both pyrimidine nucleosides is much more stable than that of the purines, and thus the pyrimidines tolerate incubation with Lewis acids better. As expected, incubating high concentrations of the Lewis acidic, oxidizing agents Au(I) and Ag(I) with the hexaguanine sequence **hexG-1a**, and also with the DNA sequence **hexACGT-1a** that contained all four nucleobases had disastrous consequences for the DNA (Fig S30, ESI). However, surprisingly, the hexaadenine-aldehyde conjugate **hexA-1a** tolerated the conditions of Au(I) catalysis (Table 1) and was converted to the corresponding 6-oxa-1,2-diazaspiro[4.4]nonane conjugate **hexA-4a**. We therefore designed a DNA sequence that was devoid of the nucleobase guanine, synthesized the aldehyde conjugate of this sequence **hexACT-1a**, and indeed reacted it successfully to the spirocycle **hexACT-4a**.

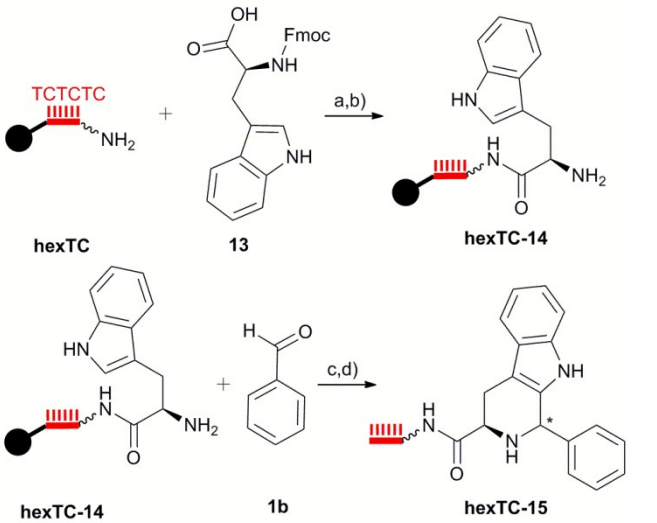


Fig. 5 Synthesis of a hexTC-tagged β-carboline **hexTC-15** from the corresponding tryptophane conjugate **hexTC-14** by trifluoroacetic acid-catalyzed Pictet-Spengler reaction. Reagents and conditions : a) HATU, DIPEA, DMF, room temperature, 4 hours; b) piperidine, DMF; c) 2 % trifluoroacetic acid, dichloromethane, room temperature, 18 hours; d) aq. $NH_3/MeNH_2$, room temperature, 30 min. Wavy bond to hexTC: 5'-(C6)-amino-linker, bold bond: connection to solid phase, filled circle: solid phase.

We attribute the differential stability of the adenine- versus the guanine-containing sequences under conditions of the Au(I)-mediated reaction to the lower redox potential of guanine versus adenine,[14] and the higher susceptibility of guanine to electrophilic attack, which is documented e.g. for the reaction of cisplatin with DNA.[15]

Having established that both pyrimidine nucleobases were stable under the conditions of the Au(I)-mediated spirocyclization reaction, we revisited the previously published hexT-compatible Au(I)-mediated annulation reaction yielding highly substituted pyrazol(in)es (Fig. 1B), and the acid-catalyzed Pictet-Spengler reaction.[11] Coupling Fmoc-protected tryptophan to the 5'-aminolinker modified **hexTC** yielded **hexTC-14** which was *N*-deprotected on solid support, and reacted with benzaldehyde **1b** under catalysis by the strong protic acid TFA. Gratifyingly, the reaction furnished the target β-carboline **hexTC-15** (Figs 5, and S31/32 ESI). In a further experiment *para*-ethynylbenzoic acid **16** was coupled to hexTC furnishing the conjugate **hexTC-17**.

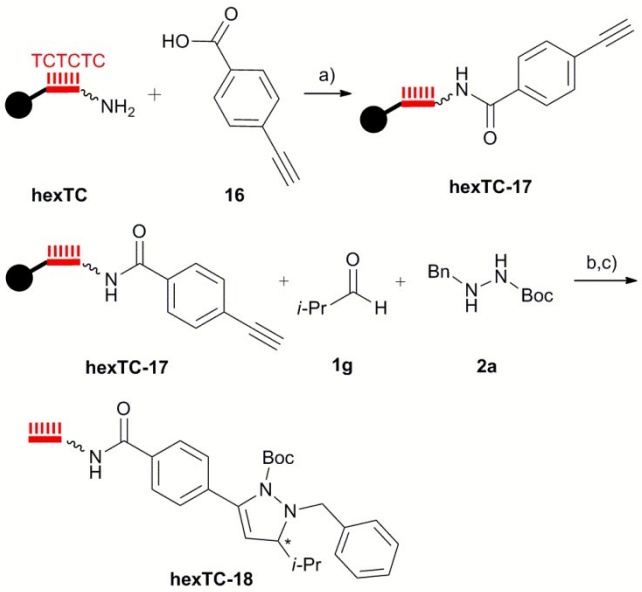


Fig. 6 Synthesis of a hexTC-tagged pyrazoline **hexTC-18** from the alkyne conjugate **hexTC-17**, isobutyraldehyde **1g** and the *N*-benzyl- and *N´*-Boc-substituted hydrazide **2a** by a Au(I)-mediated annulation reaction. Reagents and conditions: a) HATU, DIPEA, DMF, room temperature, 4 hours; b) 30 nmol scale, 250 eq. tris(2,4-di-*tert*-butylphenyl)phosphite]gold chloride, 250 eq. $AgSbF_6$, 1000 eq. **1g**, 1000 eq. **2a**, MeCN, 50°C, 20 hours; c) aq. NH_3/$MeNH_2$, room temperature, 30 min. Wavy bond to hexTC: 5'-(C6)-amino-linker, bold bond: connection to solid phase, filled circle: solid phase.

This conjugate reacted smoothly with isobutyraldehyde **1g** and the *N*-benzyl- and *N´*-Boc-substituted hydrazide **2a** by a Au(I)-mediated annulation reaction to the highly substituted pyrazoline **hexTC-18 (**Figs 6, and S31/33 ESI).[11]

Conclusions

Expanding the chemical space of genetically tagged screening libraries of small organic molecules is currently an important goal in the field. One approach to such libraries uses a chemically very stable hexathymidine adapter oligonucleotide "hexT" which is connected to coding DNA sequences following small molecule synthesis.[11] Here, we demonstrate the synthesis of hexathymidine DNA conjugates of the 6-oxa-1,2-diazaspiro[4.4]nonane scaffold, a fully saturated, highly substituted spirocyclic structure, from simple, readily available aldehydes, hydrazides, and alkynols by a Au(I)-mediated reaction. Reactions that employ three or even more reactants offer the opportunity to append any one of these to the DNA, thereby varying the connectivity between small molecule and tag. We appended any of the three reactants required for synthesis of the target 6-oxa-1,2-diazaspiro[4.4]nonane to the hexT adapter oligonucleotide, and optimized reaction conditions for product formation. However, it turned out that the DNA-aldehyde conjugate **hexT-1a** was the only viable option for library synthesis, yielding the target spirocycle with minimal formation of side products (Fig. 2). The corresponding reaction employing the hydrazide conjugate **hexT-2d** returned a complex product mixture (Fig. 3) due to massive formation of degradation and side products. Also, the alkynol **hexT-3c** gave the target product in low yields, mainly due to addition of water to the intermediate cyclic enol ether,[13] and as a mixture making tedious purification necessary which precludes parallel synthesis of larger numbers of compounds. Next, several DNA sequences were tested for their compatibility with the Au(I)-mediated reaction. While both pyrimidine bases tolerated the reaction conditions, the guanine-containing sequences were degraded to a degree that precluded product analysis. Surprisingly, we found the adenine-containing hexA, and consequently the sequence hexATC sufficiently stable to allow for product isolation. In conclusion, this study prompts an investigation in the tolerance of the four DNA nucleobases to diverse reaction conditions, which may eventually be exploited by the design of novel DNA-tagging strategies.

Conflicts of interest

There are no conflicts to declare.

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