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# Synthesis of an adenine N-3 substituted CBI adduct by alkylation of adenine with a 1-iodomethylindoline seco-CBI precursor

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ABSTRACT

The synthesis of a 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[e]indol-4-one (CBI)-adenine adduct via regioselective N-3 alkylation of adenine with a 1-(iodomethyl)-2,3-dihydro-1*H*-benzo[e]indol-5-ol (I-seco-CBI)-containing precursor is described. Spectroscopic analyses of the unlabeled and adenine-C8 carbon-13 labeled adducts utilizing ROESY NMR techniques allowed structural assignment of the alkylation product as the adenine N-3 substituted regioisomer. A stable-isotopically labeled version of the N-3 adduct incorporating six carbon-13 labels was also prepared by this method for use as an LC-MS internal standard. The cyclized CBI-containing compound was also found to alkylate adenine at elevated temperatures to produce the N-3 adduct albeit at a significantly slower rate than that observed for the I-seco-CBI precursor. Adenine alkylation with an I-seco-CBI precursor offers scalable and facile access to a CBI-adenine adduct facilitating its use as an efficacy marker for the development of duocarmycin-based ADCs.



## 1. Introduction

Duocarmycins and related analogs are well known highly potent antitumor agents [1–3]. The known myelotoxicity and hepatotoxicity of these drug molecules significantly narrows the therapeutic window for their use as chemotherapy drugs [4,5]. In an attempt to overcome these therapeutic limitations, duocarmycin-related compounds have recently served as payloads in antibody-drug conjugates (ADCs) utilizing targeted delivery to enhance their tumor killing ability while avoiding the undesired toxicity associated with these compounds [6–13].

The mechanism of cytotoxicity of the duocarmycins and related analogs has been extensively investigated through the work of Boger [14–18] and others [19–22]. This putative mechanism involves initial minor groove binding of duocarmycins to adenine-rich DNA. Molecular shaping enhances the electrophilicity of the cyclopropyl moiety allowing regioselective alkylation of the N-3 of adenine on the DNA resulting in tumor cell death through apoptosis [23]. Subsequent degradation of modified DNA affords adducts which confirm that the adenine base on DNA is modified selectively at the N-3 position [15,24].

## 2. Results and discussion

### 2.1. Synthesis

As part of bioanalytical assays to support development of ADCs possessing a 1-(chloromethyl)-2,3-dihydro-1*H*- benzo[e]indol-5-ol (seco-CBI)-containing payload component, measuring levels of N-3 adenine adduct **1** (Fig. 1) could serve as a valuable tool to evaluate drug efficacy in preclinical *in vivo* models, and as a potential clinical biomarker [25]. However, execution of this assay required substantial amounts of **1** for use as a reference standard, as well as an isotopically labeled analog of **1** for use as an LC-MS internal standard. Previously reported preparations of 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[e]indol-4-one (CBI)-adenine N-3 adducts have required lengthy incubations of CBI derivatives with adenine-rich calf thymus DNA, followed by depurination, extraction and preparative chromatography to isolate the desired adduct from the

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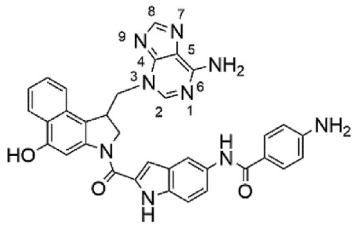


1

**Fig. 1.** N-3 Adduct **1** with numbered adenine positions.

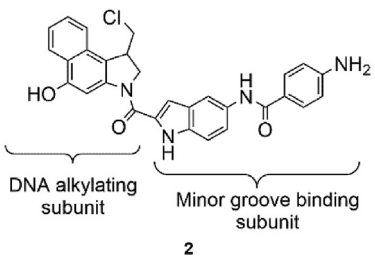
degradation mixtures [15,24]. This approach while well refined, is not amenable to scale-up, or to modification for the preparation of isotopically labeled analogs. No previous synthetic approach to these adducts using direct alkylation of adenine with a CBI or seco-CBI related precursor has been reported. We now describe the synthesis of the CBI-adenine adduct **1** and its isotopically labeled analog via N-3 alkylation of adenine with an iodo-substituted seco-CBI precursor.

The S-enantiomer of **2** (Fig. 2) was recently developed as the seco-CBI-containing payload component of a CD70 targeted ADC [26,27] and was previously shown to have enhanced cytotoxicity in comparison to naturally occurring duocarmycins [28]. Compound **2** is comprised of a seco-CBI alkylating subunit linked to a DNA binding minor-groove component.

To investigate conditions allowing reaction of the alkylating subunit in **2** with adenine in solution to form the desired adduct **1**, we prepared the racemic seco-CBI precursor **5** (Scheme 1). Coupling of the known racemic chloride **3** [22] with N-Boc-protected 4-aminobenzoic acid using HATU activation readily afforded the known seco-CBI precursor **5** [28] in high yield. The racemic material was used due to the ready availability of racemic starting material **3** and because all planned bioanalytical assays measuring adduct levels utilized non-chiral LC-MS assays.

While CBI-containing compounds rapidly and regioselectively react with the N-3 position of adenine in DNA [15], CBI and closely related ring systems undergo slow solvolysis [29] and are unreactive to adenine in solution [30]. Therefore, we investigated alkylating adenine regioselectively with a potentially more reactive seco-CBI precursor using halogen substitution chemistry. Adenine can behave as an ambident nucleophile and regioselective N-3 alkylation by alkyl halides has been shown by Leonard to require elevated temperatures and polar solvents such as DMF or DMA favoring ionic character [31]. These polar, hydrophilic solvents are also required for appropriate solubility of adenine in reaction mixtures.

Treatment of chloride **5** with excess adenine in DMA at 80 °C gave only trace amounts of adenine-containing adducts (as identified by LC-MS) even after extended heating (72 h). To enhance reactivity, chloride **5** was converted to the corresponding iodide **6**




2

**Fig. 2.** Seco-CBI-containing payload **2**.

(Scheme 1) using Finkelstein reaction conditions in butanone at 78 °C. Iodide **6** was found to have sufficient stability from cyclization to the CBI system allowing workup and characterization by LC-MS and NMR analyses. Difficulties in efficiently isolating pure **6** coupled with the low reactivity of chloride **5** prompted us to utilize the crude iodide (containing approximately 10% of chloride **5**) directly in subsequent reactions. Treatment of iodide **6** with five equivalents of adenine in DMA at 80 °C for 18 h resulted in complete consumption of the starting material. LC-MS analyses of the resulting product mixture showed approximately 40–50% of adenine-containing adducts along with 20–30% of the cyclized CBI system **7** and 5–10% of unreacted **5** which had been carried through from the previous step. Further HPLC and LC-MS evaluations of the adenine-containing components established the presence of approximately 70% of one major adenine isomer in the mixture with the observed molecular ion of *m*/*z* 710 in the mass spectrum which was later identified as the adenine N-3 adduct. The observed regioselectivity towards this isomer was consistent with previously observed N3-alkylations of adenine by alkyl halides [31]. Three other adenine adducts with the same molecular ion of *m*/*z* 710 were also observed as minor components including one adduct (presumably the N-9 adenine substituted adduct [31]) comprising approximately 14% of the adduct mixture. Isolation of the major adenine-adduct by semi-preparative HPLC followed by acidic removal of the *N-Boc* protecting group afforded the pure adduct **1b** in 24% isolated yield in two steps from chloride **5**. This isolated compound was found to co-elute on HPLC with a reference sample of the R-enantiomer of adduct **1b** derived from incubations of the R, S-enantiomer of **7** with calf thymus DNA using literature procedures [15].

### 2.2. Structural identification

Spectroscopic analyses utilizing 2D NMR techniques provided further confirmation of the structural assignment of **1b** as the adenine N-3-substituted isomer. Boger has described 2D $^{1}$H-$^{1}$H NOESY NMR experiments in which diagnostic NOEs between both the H-13 protons of the side-chain and the H-2 proton of the adenine were observed exclusively in adenine N-3 substituted adducts (Fig. 3) [15]. In our hands, strong NOE crosspeaks were observed in the 2D $^{1}$H-$^{1}$H ROESY between the upfield adenine proton H-2 at 7.68 ppm, and the $CH_2$-13 protons (5.05 and 4.75 ppm) confirming the N3-substituted adenine structure **1b**.

While the assignment of the upfield singlet (relative to the other adenine proton at 8.11 ppm) to the adenine C2-H position was consistent with spectra from related adenine adducts [15], the key chemical shift differed from the literature values, most likely due to pH differences in the sample and the effects of modified substituents. In order to unambiguously confirm the adenine proton assignment and the isomeric structure, we synthesized the carbon-13 labeled analog **1d** (Scheme 2). This was accomplished via a reaction of iodide **6** with adenine labeled with carbon-13 at the C8 position followed by acidic deprotection of the resulting adenine *N-Boc* protected adduct **1c**. It was expected that the large coupling constant between the carbon-13 labeled adenine C8 and the H-8 proton would readily differentiate this proton from the H-2 proton in the spectra. As displayed in Fig. 3 the ROESY spectrum of **1d** shows the absorbance associated with adenine H-8 as the expected doublet possessing a large $^{13}$C-H coupling (8.3, 7.90 ppm, d, $J = 220.8$ Hz). The other proton associated with adenine (H-2) remains as a singlet at 7.68 ppm. Furthermore, the downfield component of the doublet assigned to H-8 at 8.32 ppm does not overlap with any other absorbances in the spectrum, and clearly shows no observable NOE correlations with other protons in the spectrum. This lack of NOE is consistent with the distal positioning

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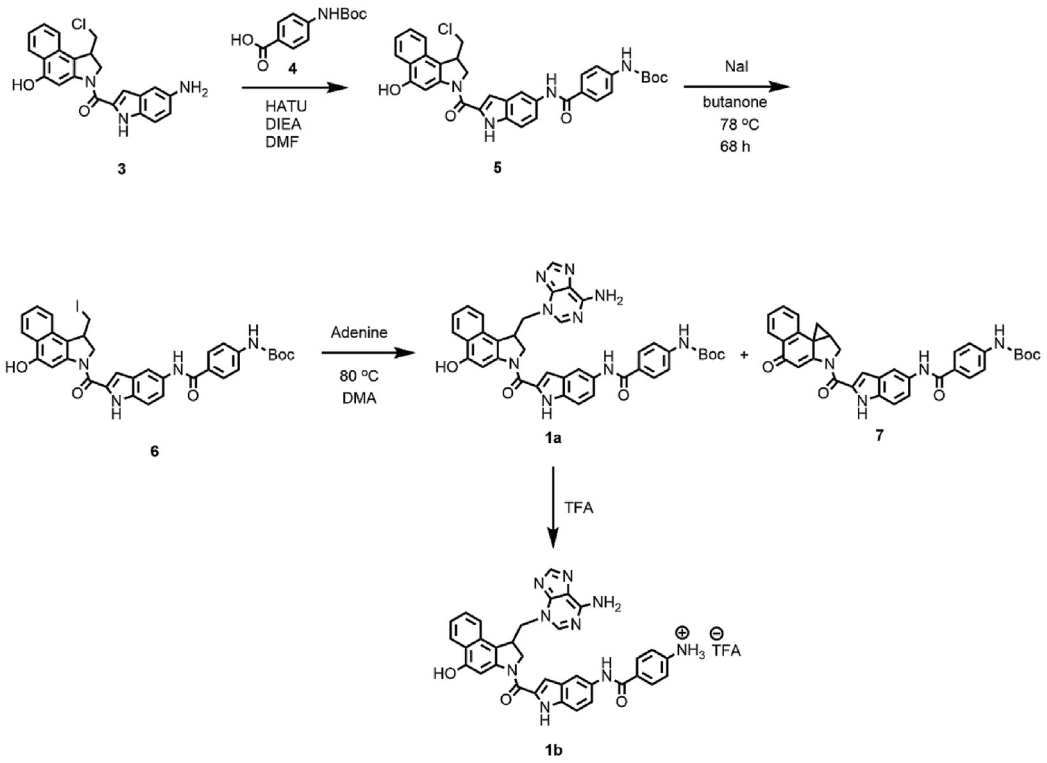


**Scheme 1.** Synthesis of iodide **6** and its reactions with adenine.

of H-8 to the H-13 methylene protons and confirms the N3-adenine adduct structure assigned as **1d**.

### 2.3. Synthesis of stable-isotopically labeled *1* for use as an LC-MS internal standard

With confirmation that the isolated product was in fact the desired N-3 adenine adduct, we applied the identical sequence to the synthesis of the $C_6$ carbon-13 labeled analog **1f** (Scheme 3) for use as an LC-MS internal standard in bioanalytical assays. This approach allowed facile isotope labeling in the benzoic ring system of **1**. Commercially available *N*-Boc-*p*-amino benzoic acid **4b** containing six carbon-13 isotopic ring labels was coupled to indole amine **3** to give **5b** in 48% yield. Following conversion to the iodide (**6b**) and reaction with adenine, **5b** was converted to the stable-labeled adenine adduct **1e** in 28% yield from chloride **5b**. The *N*-Boc group was then removed under standard conditions to afford **1f**. With possession of six isotopic mass units, adduct **1f** was well suited for use as an LC-MS internal standard for adduct bioanalytical assays with minimal interference or cross signal contribution with unlabeled analyte during MS based quantifications.

### 2.4. Further investigation of the adenine alkylation

During our investigation of adenine alkylation with iodide **6**, formation of the ring-closed CBI **7** was consistently observed as 20–30% of the product mixture. The possibility therefore exists that the observed alkylation of adenine is proceeding via initial cyclization of iodide **6** to the CBI **7** which could then alkylate adenine in a similar manner to the mechanism seen in DNA alkylation. Free CBIs are known as very weak electrophiles in solution [29] and structurally similar cyclopropylpyrroloindoles have been reported to have no reaction with excess adenine in DMF for extended heating at 45 °C [30]. However, the elevated temperature (80 °C) associated with the adenine alkylation conditions of iodide **6** may enable **7** to react with adenine. In order to investigate this possibility, we prepared the known CBI-containing compound **7** [32] (Scheme 4) via treatment of chloride **5** with DBU under established conditions [15]. Treatment of **7** with excess adenine at 82 °C showed slow formation of the N-3 adduct **1a** with side-products. After 72 h reaction time, HPLC-UV and LC-MS analyses showed a product mixture consisting of 59% adenine-containing adducts as an approximate 4:1 mixture of N-3 substituted adduct and a single additional adduct (presumably the adenine N-9 substituted). Also observed as 26% of the mixture was the hydrolyzed product **8** from adventitious water in the reaction, and 14% of unreacted starting material **7**. Unlike the profile from adenine alkylation with iodide **6** in which four adenine-related isomers were observed, only two adenine-related adducts were seen in the mixture. This difference in isomeric mixture suggests sluggish reactivity of **7** with weakly basic adenine compared to the seco-CBI iodide **6**. While this result demonstrates that at appropriately elevated temperatures, free CBI systems can alkylate adenine in solution, the observed differences in alkylation rates (18 h versus 72 h) and product profiles, provide evidence that the primary pathway of adenine alkylation by iodide **6** does not include initial cyclization of **6** to the CBI **7**.

In an attempt to block undesired cyclization of iodide to the CBI during adenine alkylation, we protected the hydroxyl group in **6** with several protecting strategies. Initial attempts protected the 4-hydroxyl in **6** with bulky silyl protecting groups. However, silyl ethers were found to be unstable to the alkylation conditions. We then turned to methylpiperazine carbamate as a known protecting group for seco-CBI systems [26,32]. Carbamate protected iodide **10** was readily prepared via acylation of **6** with methylpiperazine acetyl chloride affording **10** in 40% yield after purification (Scheme 5). After heating iodide **10** with 5 equivalents of adenine at 80 °C for 24 h, approximately 35% of the starting material remained and the product mixture was comprised of approximately 14% adenine-

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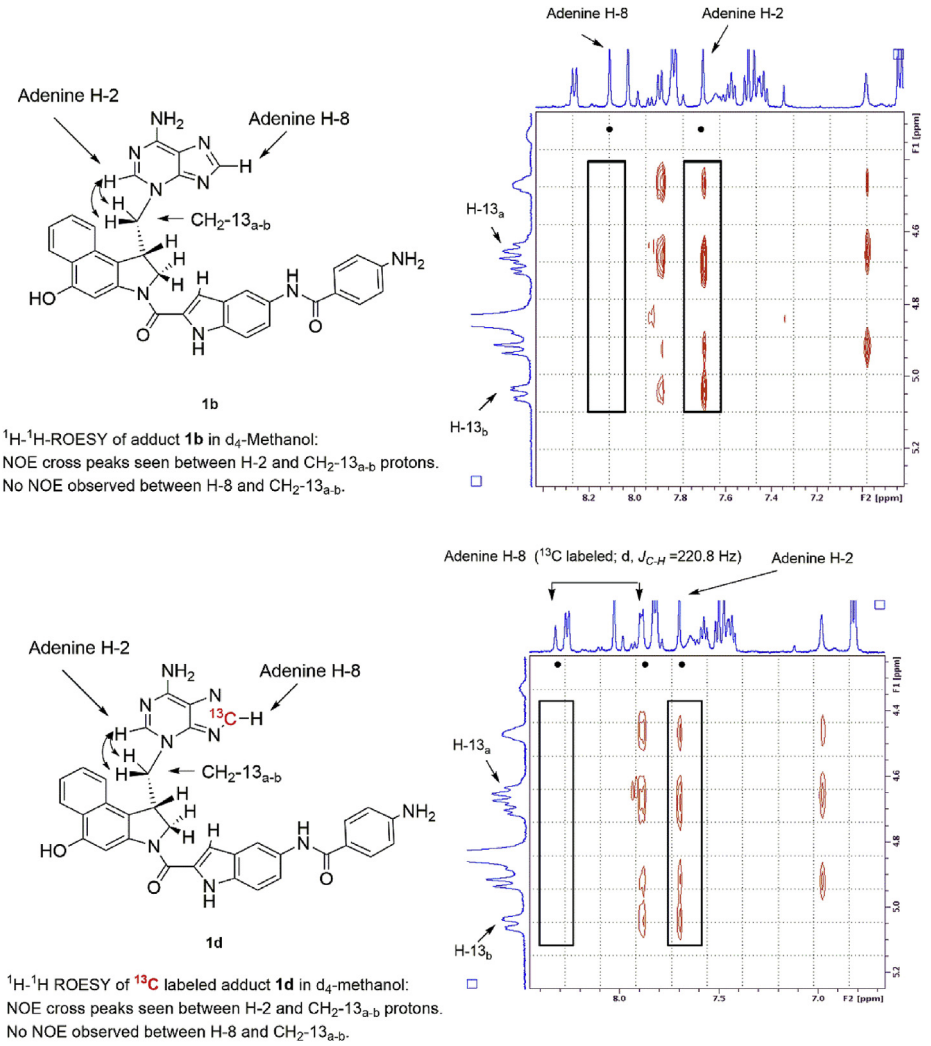
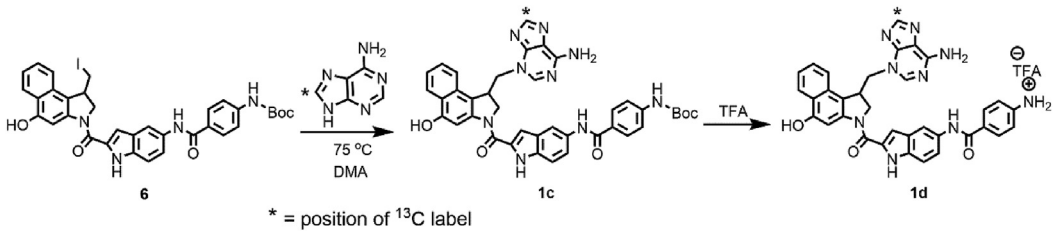



**Fig. 3.** ROESY NMR analysis of adenine adduct **1b** and $^{13}$C labeled adduct **1d**.




**Scheme 2.** Synthesis of adenine C8-carbon-13 labeled adduct **1**.

adducts **11a** by HPLC analysis. These adducts showed a similar ratio of the four isomers as observed previously in reactions of iodide **6** with adenine. Also identified in the product mixture were substantial amounts of compound **11b (**presumably resulting from hydrolysis with adventitious water), and a non-adenine containing product **12.** Continued heating for a total of 48 h showed 10% of iodide **10** still remaining in the product mixture while levels of adenine-containing adducts (**11a)** rose only slightly. However, the hydrolysis product **11b** was no longer observed after 48 h and **12** had become the major product comprising approximately 40% of the mixture. Semi-preparative HPLC afforded pure product **12** in 30% yield which was structurally identified by MS and NMR analyses as **12**: resulting from elimination and internalization of the resulting double bond. Surprisingly, the carbamide modification appeared to diminish observed iodide substitution with adenine, and allowed side-reactions such as iodide substitution with water to become substantial reaction pathways. The observed initial accumulation of the hydrolysis product **11b** followed by its disappearance and corresponding increase of **12** suggests that it is in fact the hydroxyl compound **11b** (not the iodide **10**) which primarily undergoes elimination and double bond migration to produce **12**. While the scope of this study focused on reaction conditions precedented to favor N-3 adenine alkylation [31], future work could explore alternative conditions using base or other additives to enhance alkylation at lower temperatures and suppress formation of the cyclized CBI side product.

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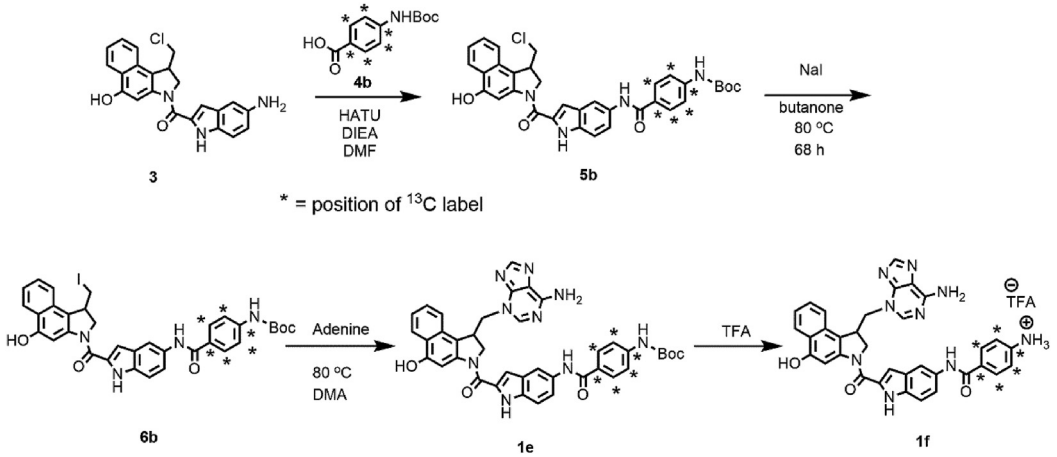
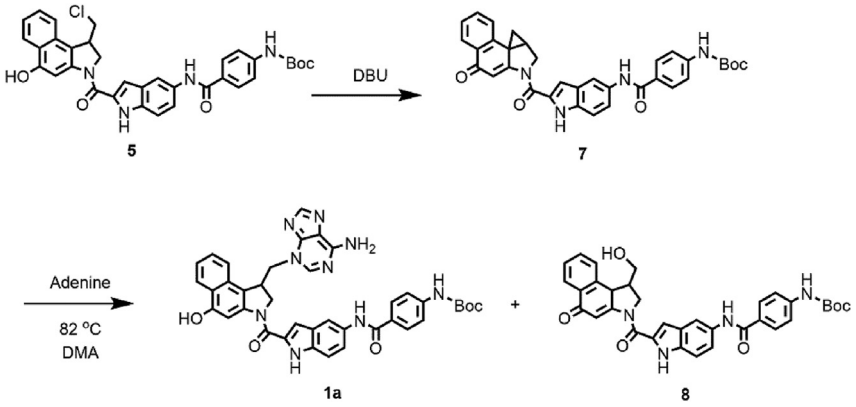
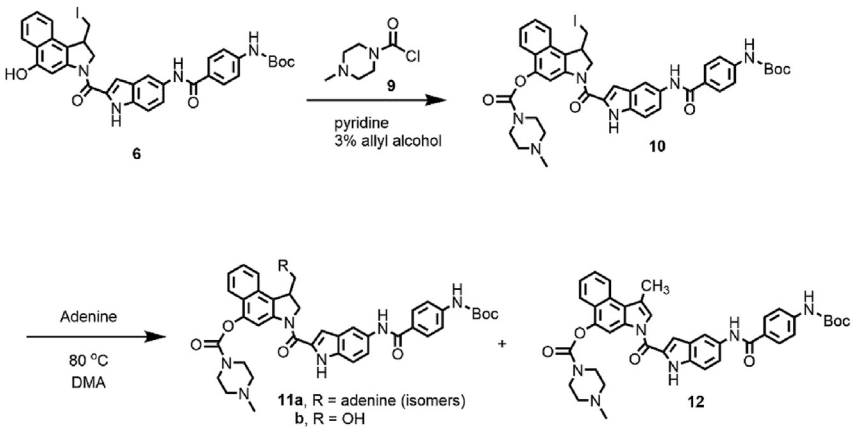



**Scheme 3.** Synthesis of the $^{13}C_6$ labeled internal standard **1f**.




**Scheme 4.** Preparation of CBI-containing **7** and its reaction with adenine at elevated temperatures.




**Scheme 5.** Preparation of carbamate 10 and its reaction with adenine.

## 3. Conclusion

A procedurally facile method for synthesizing CBI-adenine adducts via alkylation of adenine in solvent with iodo-substituted seco-CBI precursors has been developed. These alkylation conditions should be applicable to other CBI and related ADC payload systems in which DNA alkylation is a therapeutic target. Furthermore, this method is applicable to the synthesis of stable-isotopically labeled analogs of these adducts which serve as internal standards for mass spectrometry based efficacy and biomarker assays in the development of duocarmycins and related ADCs.

## 4. Experimental

### 4.1. General information and methods

All unlabeled reagents were of ACS grade or better. Racemic (5-

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amino-1*H*-indol-2-yl)(1-(chloromethyl)-5-hydroxy-1*H*-benzo[e] indol-3(2H)-yl)methanone (**3**), TFA salt was provided by Bristol-Myers Squibb Chemical Development. Adenine (8–$^{13}$C) was purchased from Cambridge Isotope laboratories Inc. (Tewksbury, MA). [$^{13}C_6$]4-((tert-Butoxycarbonyl)amino)benzoic acid (**4b**) was purchased from IsoSciences Inc. (King of Prussia, PA). Samples of the N-3 adenine adduct **1b** standard prepared from incubations of the (R,S)-enantiomer of **7** with calf thymus DNA [15] were provided by Bristol-Myers Squibb Biologics Discovery California (Redwood City, CA). Reactions were run under an inert atmosphere of nitrogen and magnetically stirred at a constant rate. Semi-preparative HPLC purifications were performed using a Varian Chromatography system. Analytical HPLC analyses were performed on a Shimadzu model SCL-10A system with an SPD-10AV UV–vis detector. LC-MS analyses were performed on an Agilent 1100 series HPLC and a Finnigan LXQ ion trap mass spectrometer (Desolvation Gas: Nitrogen; Capillary Temp: 270 °C; Positive Electrospray). High resolution mass spectra (HRMS) were obtained using a Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (with electrospray ionization (ESI) source) that was connected to a Shimadzu Nexera UPLC system (Cortecs UPLC C18 column, 1.6 μ (2.1 × 50 mm)). Reactions were monitored by HPLC and LC-MS with comparisons made to authentic unlabeled materials when available. Melting points were obtained using an EZ-Melt Automated melting point apparatus (Stanford Research Systems).

**NMR**. Proton NMR spectra were recorded on a Bruker 300 MHz Avance II NMR spectrometer with a 5 mm $^{3}$H/$^{1}$H/$^{13}$C QNP probe at 27 °C. One and two-dimensional NMR spectra for structure elucidation purposes were collected on either a Bruker 500 MHz Avance III NMR Spectrometer with a 5 mm Bruker BBFO+ 500 probe at 27 °C, a Bruker 500 MHz Avance III NMR Spectrometer with a 5 mm Bruker BBO Prodigy 500 cyroprobe at 27 °C, or a Bruker 600 MHz NEO NMR Spectrometer with a 5 mm Bruker TCI cryo probe at 27 °C.

**LCMS**. LC-MS methods described below were used for in-process and final product analyses.

*Method 1*: Column: Luna C18(2), 3.0 × 100 mm, 3 μm particle size. Flow rate: 0.5 mL/min. Mobile phase A: water, 0.1% formic acid; Mobile phase B: acetonitrile, 0.1% formic acid, linear gradient of 20% B to 80% B over 13 min.

*Method 2*: Column: Luna C18(2), 3.0 × 100 mm, 3 μm particle size. Flow rate: 0.5 mL/min. Mobile phase A: water, 0.1% formic acid; Mobile phase B: acetonitrile, 0.1% formic acid, linear gradient of 20% B to 95% B over 7 min.

**HPLC**. HPLC methods described below were used for in-process and final product analyses.

*Method 3*: Column: Luna, 4.6 × 150 mm, 5 μm particle size. Mobile phase A: water with 0.1% TFA. Mobile phase B: $CH_3CN$. Linear gradient of 30% B to 100% B over 20 min; Flow rate: 1 mL/min, injection volume: 10 μL, diluent: $CH_3CN$; UV Detection: λ = 220 nm.

***Semi-preparative HPLC***. HPLC methods described below were used for purifications.

*Method 4*: Column: Phenomenex Luna C18 (2), 21.2 × 250 mm, 5 μm particle size; Mobile Phase A: aqueous 0.1% TFA; Mobile Phase B: Acetonitrile; Gradient: 55–85 over 30 min, flow rate: 10 mL/min; UV Detection: λ = 254 nm.

*Method 5*: Column: Phenomenex Luna C18 (2), 21.2 × 250 mm, 5 μm particle size; Mobile Phase A: aqueous 0.1% TFA; Mobile Phase B: Acetonitrile; Gradient: 20–80 over 20 min, flow rate: 10 mL/min; UV Detection: λ = 254 nm.

*Method 6*: Column: Eclipse XDB-C8 column, 21.2 × 250 mm, 5 μm particle size; Mobile Phase A: aqueous 0.1% TFA; Mobile Phase B: Acetonitrile; Gradient: 20–100% over 30 min, flow rate: 10 mL/min; UV Detection: λ = 254 nm.

*Method 7*: Column: Phenomenex Luna C18 (2), 21.2 × 250 mm, 5 μm particle size; Mobile Phase A: aqueous 0.1% TFA; Mobile Phase B: Acetonitrile; Gradient: 30–100 over 20 min, flow rate: 10 mL/min; UV Detection: λ = 254 nm.

### *4.2. tert-Butyl(4-((2-(1-(chloromethyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)carbamoyl)phenyl) carbamate (**5**)*

To a solution of 4-((tert-butoxycarbonyl)amino)benzoic acid (**4**, 196 mg, 0.825 mmol) in DMF (5 mL) was added 2-(3H-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (HATU, 314 mg, 0.825 mmol) followed by *N,N*-diisopropylethylamine (0.432 mL, 2.48 mmol). The resulting solution was stirred for 45 min at ambient temp. The solution was then added to another flask containing a solution of (5-amino-1*H*-indol-2-yl) (1-(chloromethyl)-5-hydroxy-1*H*-benzo[e]indol-3(2H)-yl) methanone [22], TFA salt (**3**, 500 mg, 0.990 mmol) dissolved in 5 mL of DMF. The flask containing the solution of activated **4** was rinsed with 200 μL of DMF which was added to the reaction. To the flask was then added Hünig's base (0.451 mL, 2.6 mmol) and the resulting dark, uniform solution was allowed to stir at ambient temp. After 45 min, a sample was dissolved in acetonitrile and analyzed by LC-MS (method 1). This analysis showed the formation of a new product (Tr = 7.0 min) with $m/z = 611$ which is the expected ion for the product. To the solution was added 0.5 N HCl (55 mL) causing a precipitate to form. After stirring for 1 h, the precipitate was collected by vacuum filtration on a fritted funnel. After air drying for 10 min, the funnel was dried under vacuum at ambient temp for 12 h to afford crude **5** [22] as a greenish-grey powder: $^{1}$H NMR (DMSO-$d_6$): δ 11.69 (s, 1 H), 10.42 (s, 1 H), 10.02 (s, 1 H), 9.66 (br s, 1 H), 8.17 (br s, 1 H), 8.14 (d, 1 H, $J$ = 8.36 Hz), 7.99 (br s, 1 H), 7.95 (d, 2 H, $J$ = 8.8 Hz), 7.87 (d, 1 H, $J$ = 8.36 Hz), 7.61 (d, 2 H, $J$ = 8.8 Hz), 7.56 (dd, 1 H, $J$ = 8.8, 1.9 Hz), 7.53 (d, 1 H, $J$ = 7.04 Hz), 7.47 (d, 1 H, $J$ = 8.8 Hz), 7.39 (br t, 2 H, $J$ = 7.04 Hz), 7.21 (s, 1H), 4.82 (br t, 1 H, $J$ = 11 Hz), 4.58 (br dd, 1 H, $J$ = 11, 1.8 Hz), 4.25 (m, 1 H), 4.04 (m, 1 H), 3.89 (dd, 1 H, $J$ = 11, 7.3 Hz), 1.51 (s, 9 H); mp 131–135 °C (dec).

### *4.3. tert-Butyl(4-((2-(1-((6-amino-3H-purin-3-yl)methyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)carbamoyl)phenyl)carbamate (**1a**)*

To a solution of **5** (150 mg, 0.245 mmol) in 2-butanone (10 mL) was added sodium iodide (184 mg, 1.23 mmol) and the resulting mixture was stirred at 78 °C under nitrogen and monitored by HPLC (method 3, compound **6,** Tr = 15.1 min, chloride **5**, Tr = 14.8 min). After heating for 72 h conversion of **5** to the corresponding iodide **6** was approximately 90% complete. After cooling, the mixture was evaporated to dryness *in vacuo,* and the residue partitioned between water and ethyl acetate. The layers were separated, the aqueous layer extracted twice with ethyl acetate, and the combined organic layers were washed with brine and then dried over magnesium sulfate to give 170 mg of crude **6**: LC-MS analysis (method 2) of the solution (compound **6**, Tr = 5.6 min) showed $m/z$ $[M+H]^+$ calcd for $C_{34}H_{31}IN_4O_5$ 703.13, found 703.12. This material was stored as a solution in EtOAc at 4 °C and was evaporated to give a solid residue as required for the next step.

A 12 mg portion of crude **6** was subjected to semi-preparative HPLC (method 4) which afforded purified **6** (6 mg, 0.01 mmol) used for characterization: HRMS $[M+H]^+$ calcd for $C_{34}H_{31}IN_4O_5$ 703.1412, found 703.1405; $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 11.67 (br d, $J$ = 1.3 Hz, 1H), 10.02 (s, 1H), 9.66 (s, 2H), 8.14 (d, $J$ = 1.3 Hz, 1H), 8.10 (d, $J$ = 8.5 Hz, 1H), 7.98 (br s, 1H), 7.92 (br d, $J$ = 8.8 Hz, 2H), 7.80 (d, $J$ = 8.2 Hz, 1H), 7.59 (br d, $J$ = 8.8 Hz, 2H), 7.55 (br dd, $J$ = 9.0,

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2.0 Hz, 1H), 7.50 (td, $J = 7.6$, 1.1 Hz, 1H), 7.46 (d, $J = 8.8$ Hz, 1H), 7.36–7.30 (m, 1H), 7.20 (d, $J = 1.6$ Hz, 1H), 4.72–4.65 (m, 1H), 4.65–4.60 (m, 1H), 3.87–3.80 (m, 1H), 3.77 (dd, $J = 10.9$, 3.9 Hz, 1H), 3.46–3.45 (m, 1H), 1.50 (m, 9H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 164.7, 160.2, 153.4, 152.6, 142.4, 141.7, 133.2, 132.0, 131.4, 130.1, 128.4 (2C), 127.0, 126.9, 123.2, 123.0, 122.1, 119.5, 117.1 (s, 2C), 116.6, 113.1, 112.0, 105.6, 100.5, 79.5, 62.9, 54.6, 42.5, 28.1 (3C), 32/34 carbons identified. Purified iodide **6** was found to convert to CBI **7** and hydrolysis product **8** on extended exposure to the acidic HPLC mobile phase.

To a solution of crude **6** (85 mg, 0.196 mmol) in *N,N*-dimethylacetamide (6.76 mL) was added 9H-purin-6-amine (82 mg, 0.605 mmol) and the resulting partial slurry was stirred under nitrogen at 80 °C for 42 h while monitored by HPLC (method 3) showing a mixture of adenine-containing adducts Tr = 11.1 min (15%), 11.8 min (N-3 substituted **1a**, 70%), 12.2 min (7%) and 12.4 min (8%) as confirmed by LC-MS analyses (method 2). Also observed by HPLC analysis (method 3) was the cyclized CBI **7** (Tr = 15.7 min, 20–30%), and unreacted chloride **5** (Tr = 14.8 min, 5–10%) which was carried over from the crude iodide starting material. The resulting dark-red solution was cooled and subjected directly to semi-preparative HPLC (method 6) which afforded 20 mg (0.028 mmol, 24% in two steps from **5**) of **1a** as a light-yellow powder: HRMS (ESI) $[M+H]^+$ calcd for $C_{39}H_{35}N_9O_5$: 710.2834, found: 710.2827.

### 4.4. 4-Amino-N-(2-(1-((6-amino-3H-purin-3-yl)methyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)benzamide TFA salt (*1b*)

Adduct **1a** (20.0 mg, 0.028 mmol) was partially dissolved into 3 mL of methylene chloride. To the mixture was added 750 μL of TFA and the resulting slurry was stirred at ambient temp for 1.5 h. After the mixture was concentrated to dryness with a stream of nitrogen, the residue was washed twice with methylene chloride. The resulting residue was dissolved in acetonitrile (1 mL) and to this solution was added 0.1% TFA in water (1 mL). This solution was then subjected to semi-preparative HPLC (method 6) to afford 11.7 mg (0.019 mmol, 67% yield) of **1b** as a yellow solid: HRMS *m/z* $[M+H]^+$ calcd $C_{34}H_{27}N_9O_3$ 610.2310, found 610.2301; $^1$H NMR (500 MHz, methanol-$d_4$) δ 8.24 (d, $J = 8.4$ Hz, 1H), 8.09 (s, 1H), 8.01 (s, 1H), 7.87 (d, $J = 8.4$ Hz, 1H), 7.81 (d, $J = 8.5$ Hz, 2H), 7.68 (s, 1H), 7.61 (d, $J = 8.7$ Hz, 1H), 7.56 (t, $J = 7.6$ Hz, 2H), 7.47 (d, $J = 11.7$ Hz, 4H), 7.43–7.39 (m, 2H), 6.96 (br. s., 1H), 6.82 (d, $J = 8.5$ Hz, 2H), 5.03 (dd, $J = 14.0$, 4.0 Hz, 1H), 4.90 (d, $J = 11.1$ Hz, 1H), 4.71–4.65 (m, 1H), 4.65–4.59 (m, 1H), 4.44 (br s, 1H); This spectrum was comparable to that observed from an authentic standard of the (R)-enantiomer of **1b** obtained from incubation of the (R,S)-enantiomer of **7** with calf thymus DNA.[15] $^{13}$C NMR (126 MHz, methanol-$d_4$) δ 167.8, 160.9, 155.1, 154.9, 152.0, 151.4, 149.7, 143.9, 142.0, 134.1, 131.5, 130.7, 130.4, 128.9 (s, 2C), 127.5, 127.3, 123.3, 123.2, 122.9, 122.3, 121.9, 120.4, 119.6, 115.1, 114.4, 113.4 (s, 2C), 111.6, 106.0, 99.9, 55.5, 52.3, 39.0. Compound **1b** was found to co-elute on HPLC (method 5, Tr = 12.7 min) and had identical LC-MS properties (method 1) with an authentic standard of the (R)-enantiomer of 1b.

### 4.5. tert-butyl(4-((2-(1-((6-amino-8[$^{13}$C]-3H-purin-3-yl)methyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)carbamoyl)phenyl)carbamate (*1c*)

To a solution of crude iodide **6** (85 mg, 0.121 mmol, containing approximately 10% of chloride **5** as an impurity) in dimethylacetamide (5 mL) was added 8-[$^{13}$C]-9*H*-purin-6-amine (82 mg, 5 eq). The mixture was placed under nitrogen and heated at 75 °C for 18 h with monitoring of the reaction progress by HPLC (method 3, product Tr = 12.2 min). After cooling, the resulting crude mixture was directly purified by semi-preparative HPLC (method 6) which afforded 16 mg of purified **1c** (0.023 mmol, 25% in two steps from chloride 5) as a light-yellow solid: LC-MS *m/z* $[M+H]^+$ calcd for $C_{38}^{13}CH_{35}N_9O_5$: 711.28, found: 711.28.

The mixture was placed under nitrogen and heated at 75 °C for 18 h with monitoring of the reaction progress by HPLC (method 3, product Tr = 12.2 min). After cooling, the resulting crude mixture was directly purified by semi-preparative HPLC (method 6) and afforded 16 mg (0.023 mmol, 25% in two steps from chloride **5**) of purified **1c** as a light-yellow solid: LC-MS *m/z* $[M+H]^+$ calcd for $C_{38}^{13}CH_{35}N_9O_5$: 711.28, found: 711.28.

### 4.6. 4-((2-(1-((6-amino-8-[$^{13}$C]-3H-purin-3-yl)methyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl) carbamoyl)benzenaminium TFA salt (*1d*)

To a partial slurry of **1c** (16 mg, in 3 mL of methylene chloride was added 750 μL of TFA. The resulting solution was stirred at ambient temp and reaction progress was monitored by HPLC (method 3, **1d** Tr = 8.3 min). After 1.5 h the mixture was concentrated and the resulting solid washed once with methylene chloride. The solid was then dissolve into a solution of 1:1 acetonitrile:0.1%TFA (2 mL) and the solution was subjected to semi-preparative HPLC (method 6) affording 9.1 mg (0.012 mmol, 53%) of pure **1d** as a light-yellow solid: $^1$H NMR (500 MHz, Methanol-$d_4$) δ 8.25 (d, $J = 8.5$ Hz, 1H), 8.01 (d, $J = 1.3$ Hz, 1H), 8.12 (d, $J = 207.8$ Hz, 1H, H-$^{13}$C), 7.88 (d, $J = 7.6$ Hz, 1H), 7.79 (d, $J = 8.8$ Hz, 2H), 7.68 (s, 1H), 7.59–7.54 (m, 1H), 7.59–7.54 (m, 1H), 7.51–7.48 (m, 1H), 7.48–7.45 (m, 1H), 7.45–7.43 (m, 1H), 7.43–7.39 (m, 1H), 6.97 (s, 1H), 6.80–6.76 (m, 2H), 5.03 (dd, $J = 13.9$, 3.8 Hz, 1H), 4.91 (br d, $J = 11.0$ Hz, 1H), 4.72–4.60 (m, 2H), 4.49–4.39 (m, 1H) (20/27 protons observed); $^{13}$C NMR (126 MHz, methanol-$d_4$) δ 169.4, 162.2, 156.5, 155. 1 (m), 153.4, 150.3, 149.0, 146.7, 146.4, 146.2 ($^{13}$C label), 135.6, 133.1, 132.2, 131.8, 130.5 (2C), 129.1, 129.0, 125.0, 124.9, 124.5, 123.9, 123.6, 122.2, 116.1, 116.0, 115.1 (2C), 113.2, 107.7, 101.4, 57.0, 53.4, 41.2; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{38}^{13}CH_{35}N_9O_5$: 611.2344, found: 611.2342.

### 4.7. tert-Butyl(4-((2-(1-(chloromethyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)carbamoyl)[ phenyl-1,2,3,4,5,6–$^{13}C_6$)carbamate (*5b*)

To a solution of [$^{13}C_6$]4-((tert-butoxycarbonyl)amino)benzoic acid (**4b**, 145 mg, 0.611 mmol) in DMF (5 mL) was added 2-(3H-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (HATU, 232 mg, 0.611 mmol) followed by Hünig's Base (0.291 mL, 1.666 mmol). The resulting solution was stirred for 1 h at ambient temp with monitoring by LC-MS (method 1, Tr = 5.5, *m/z* = 362 for the $^{13}C_6$ labeled activated ester). The solution was then added to another flask containing (5-amino-1*H*-indol-2-yl)(1-(chloromethyl)-5-hydroxy-1*H*-benzo[e]indol-3(2H)-yl)methanone [22], TFA salt (3, 281 mg, 0.555 mmol). The flask was rinsed with 200 μL of DMF which was added to the reaction. To the flask was then added Hünig's base (0.291 mL, 1.666 mmol) and the resulting dark, uniform solution was allowed to stir at ambient temp while monitored by LC-MS (method 1, **5b** Tr = 7.0 min, *m/z* = 617) The mixture was then concentrated for 2 h *in vacuo* on a rotary evaporator. Water (50 mL) was added to the residue causing a precipitate to form. After stirring for 15 min, the precipitate was collected by vacuum filtration on a fritted funnel. After air drying for 10 min, the funnel was dried under vacuum at ambient temp for 12 h to afford crude **5b** as a greenish-grey powder. Purification by flash chromatography (gradient 20:80 to 70:30 ethyl acetate:hexane [v:v]) afforded 181 mg (0.30 mmol, 48%) of pure **5b**: $^1$H NMR

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(600 MHz, Methanol-$d_4$) δ 8.24 (d, $J = 8.4$ Hz, 1H), 8.08 (s, 1H), 8.00 (s, 1H), 7.86 (br d, $J = 8.1$ Hz, 1H), 7.67 (s, 1H), 7.76 (dq, $J = 159.2$, 8.3 Hz, 2H, H-$^{13}$C), 7.55 (br t, $J = 7.5$ Hz, 1H), 7.51–7.47 (m, 1H), 7.47–7.44 (m, 1H), 7.44–7.41 (m, 1H), 7.41–7.38 (m, 1H), 6.95 (br s, 1H), 6.74 (dq, $J = 156.4$, 6.8 Hz, 2H, H-$^{13}$C), 5.02 (br dd, $J = 14.0$, 3.4 Hz, 1H), 4.90 (br d, $J = 11.0$ Hz, 1H), 4.70–4.58 (m, 2H), 4.47–4.39 (m, 1H) (20/27 protons observed); LC-MS analysis: $m/z$ $[M+H]^+$ calcd for $C_{28}^{13}C_6H_{31}ClN_4O_5$ 617.2, 619.2, found 617.5, 619.5; HRMS (ESI) $m/z$ $[M+H]^+$ calcd for $C_{38}^{13}CH_{35}N_9O_5$: 617.2257, found: 617.2244; mp 130–134 °C (dec).

### 4.8. tert-Butyl(4-((2-(1-((6-amino-3H-purin-3-yl)methyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)carbamoyl)phenyl-1,2,3,4,5,6–$^{13}C_6$)carbamate (**1e**)

To a solution of **5b** (120 mg, 0.196 mmol) in 2-butanone (5 mL) was added sodium iodide (147 mg, 0.982 mmol) and the resulting mixture was stirred at 80 °C under nitrogen and monitored by LC-MS (method 1, iodide **6b** Tr = 5.0 min, $m/z = 709.12$). After heating for 72 h conversion of **5b** to the corresponding iodide **6b** was approximately 90% complete. After cooling, the mixture was evaporated to dryness *in vacuo,* and the residue partitioned between water and ethyl acetate. The layers were separated, the aqueous layer extracted twice with ethyl acetate, and the combined organic layers dried over sodium sulfate and filtered to give a crude solution of **6b.** LC-MS analysis of the solution (method 1), $m/z$ $[M+H]^+$ calcd for $C_{28}^{13}C_6H_{31}IN_4O_5$ 709.15, found 709.12.

This material was stored as a crude solution at 4 °C and was evaporated to give a solid residue as required for the next step.

To a solution of crude **6b** (0.138 g, 0.196 mmol) in DMA (6.76 mL) was added 9H-purin-6-amine (0.132 g, 0.980 mmol) and the resulting partial slurry was stirred under nitrogen at 80 °C for 18 h while monitored by LC-MS (method 1, **1e** Tr = 5.0 min). The resulting dark-red solution was cooled and subjected directly to semi-preparative HPLC (method 6) which afforded 39.2 mg (28% in two steps from **4b**) of **1e** as a light-yellow powder: LC-MS (ESI) $m/z$ $[M+H]^+$ calcd for $C_{33}^{13}C_6H_{35}N_9O_5$: 716.30, found: 716.26; mp 260–263 °C (dec).

### 4.9. 4-((2-(1-((6-amino-3H-purin-3-yl)methyl)-5-hydroxy-2,3-dihydro-1H-benzo[e]indole-3-carbonyl)-1H-indol-5-yl)carbamoyl) benzenaminium- $^{13}C_6$ TFA salt (**1f**)

To a partial slurry of **1e** (36.2 mg, in 1 mL of methylene chloride To the slightly cloudy mixture was added trifluoroacetic acid (0.4 mL, 5.19 mmol) and the resulting uniform, light yellow solution was stirred at ambient temp for 1.5 h with monitoring by LC-MS (method 1, **1f** Tr = 2.5 min). The solution was evaporated to dryness on a rotary evaporator to afford a yellowish oil. This oil was dissolved in methanol (3 mL) and evaporated to dryness to afford **1f** (29 mg, 0.039 mmol, 76%) as a tan solid: $^1$H NMR (DMSO-$d_6$): δ 11.52 (s, 1 H), 10.51 (s, 1 H), 9.70 (s, 1 H), 9.29 (s, 1 H), 8.75 (s, 1 H), 8.59 (s, 2 H), 8.17 (d, 1 H, $J = 8.3$ Hz), 8.11 (s, 1 H), 8.05 (d, 1 H, $J = 8.3$ Hz), 7.99 (br s, 1 H), 7.74 (br d, 2 H, $J = 166$ Hz, aryl $^{13}$C-H), 7.55 (ddd, 1 H, $J = 1.1$, 8, 8 Hz), 7.52 (dd, 1 H, $J = 1.9$, 8.8 Hz), 7.49 (d, $J = 8.8$ Hz), 7.41 (ddd, 1 H, $J = 1.1$, 8, 8 Hz), 6.98 (s, 1 H), 6.64 (br d, 2 H, $J = 157$ Hz, aryl $^{13}$C-H), 4.78 (br d, 1 H, $J = 11.4$ Hz), 4.45–4.69 (m, 4 H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 160.2, 154.4, 151.8 (td, $J = 59.3$, 7.7 Hz, 1C, $^{13}$C label position), 148.0, 145.3 (br s, 1C), 142.2, 133.1, 132.5, 132.5, 131.1, 130.0, 129.1 (td, $J = 59.0$, 5.4 Hz, 2C, $^{13}$C label position), 127.5, 127.0, 127.0 (br s, 1C), 123.3, 123.2 (br s, 1C), 122.7, 122.1, 121.4 (td, $J = 58.6$, 7.3 Hz, 1C,$^{13}$C label position), 119.6, 114.3, 112.5 (td, $J = 59.7$, 5.9 Hz, 2C, $^{13}$C label position), 110.5 (br s, 1C), 105.6, 100.2, 54.4, 51.8 (br s, 1C), 39.01–38.95 (m, 1C); $^{13}$C NMR (151 MHz, Methanol-$d_4$) δ 162.3, 156.5, 155.2, 153.6 (td, $J = 59.9$, 7.6 Hz, 1C, $^{13}$C label positon), 150.3149.0, 146.4, 143.6, 135.6, 133.2, 132.2, 131.8, 130.5 (td, $J = 59.7$, 6.0 Hz, 2C, $^{13}$C label position), 129.2, 129.0, 125.0, 125.0, 124.5, 123.9, 123.7123.7 (td, $J = 58.9$, 7.6 Hz, 1C, $^{13}$C label position), 122.2, 116.2, 116.0, 114.9 (td, $J = 59.9$, 5.4 Hz, 2C, $^{13}$C label position), 107.7, 101.4, 57.0, 53.4, 41.2 (32/34 carbons observed); LC-MS (ESI) $m/z$ $[M+H]^+$ calcd for $C_{28}^{13}C_6H_{28}N_9O_3$: 616.25, found: 616.24; HRMS (ESI) $m/z$ $[M+H]^+$ calcd for $C_{28}^{13}C_6H_{28}N_9O_3$: 616.2511, found: 616.2493; mp 220–224 °C (dec).

### 4.10. 3-(5-(4-((tert-Butoxycarbonyl)amino)benzamido)-1H-indole-2-carbonyl)-1-(iodomethyl)-2,3-dihydro-1H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate (**10**)

In a 25 mL round bottom flask, crude iodide **6** (60 mg, 0.085 mmol) was dissolved into 8 mL of a 3% solution of allyl alcohol in methylene chloride, and pyridine (41.4 μL, 0.512 mmol) was added followed by 4-methylpiperazine-1-carbonyl chloride (**9**, 27.8 mg, 0.171 mmol). The resulting solution was stirred at ambient temp for 72 h while monitoring product formation by HPLC (method 3, **10** Tr = 13.0 min. After evaporation of the solvent, the resulting crude was subjected to semi-preparative HPLC purification (method 7) affording 28.1 mg (0.034 mmol, 40%) of pure **10** as a white solid: $^1$H NMR (DMSO-$d_6$): δ 11.68 (s, 1 H, N-H), 10.01 (s, 1 H, NH), 9.65 (s, 1 H, CH), 8.21 (s, 1 H, CH), 8.18 (br s, 1 H, CH), 8.00 (d, $J = 8.36$ Hz, CH), 7.92 (d, 2 H, $J = 8.8$ Hz, CH), 7.85 (d, 1 H, $J = 8.36$ Hz, CH), 7.45–7.64 (m, 4 H), 7.59 (d, 2 H, $J = 8.8$ Hz, CH), 7.23 (br s, 1 H, CH), 4.88 (dd, 1 H, $J = 9.7$, 11 Hz, $CH_2$), 4.47 (dd, 1 H, $J = 11$, 2.2 Hz, $CH_2$), 4.27 (m, 1 H, CH), 3.68–3.85 (m, 4 H), 3.49 (m, 2 H), 2.41 (m, 4 H), 2.26 (s, 3 H, $CH_3$), 1.50 (s, 9 H, $C(CH_3)_3$); HRMS (ESI) $m/z$ $[M+H]^+$ calcd for $C_{40}H_{41}IN_6O_6$: 829.2205, found: 829.2181.

### 4.11. 3-(5-(4-((tert-butoxycarbonyl)amino)benzamido)-1H-indole-2-carbonyl)-1-methyl-3H-benzo[e]indol-5-yl 4-methylpiperazine-1-carboxylate (**12**)

A screw cap test tube was charged with a solution of iodide **10** (10.8 mg, 0.013 mmol) and adenine (8.81 mg, 0.065 mmol) in 1 mL of DMA. The tube was closed and heated at 80 °C with stirring for 48 h. The progress of the reaction was monitored by HPLC (method 3) and confirmed by LC-MS (method 2) which showed adenine adducts ($m/z = 836$, 5:1 ratio presumed N-3 isomer **11a** [HPLC Tr = 10.15 min] to other adenine-containing isomers [HPLC Tr = 9.6 min, 9.9 min and 10.4 min]), hydrolysis product **11b** ($m/z = 761$, HPLC Tr = 12.3 min), starting iodide **10** ($m/z = 829$, HPLC Tr = 13.0 min) elimination product **12** ($m/z = 701$, HPLC Tr = 13.2 min). After 48 h the reaction showed approximately 10% of the starting iodide 10 remaining and the mixture was allowed to cool and to the solution was added 0.1% aqueous TFA (100 μL). This acidified mixture was subjected to semi-preparative HPLC to isolate **12** (3.6 mg, 40%) as a white solid: $^1$H NMR (DMSO-$d_6$): δ 12.12 (s, 1 H, N-H), 10.07 (s, 1 H, NH), 9.66 (s, 1 H, N-H), 8.57 (d, 1 H, $J = 8.6$ Hz, CH), 8.32 (s, 1 H, CH), 8.24 (br s, 1 H, CH), 8.05 (s 1 H, CH), 8.03 (br d, 1 H, $J = 8.36$ Hz, CH), 7.92 (d, 2 H, $J = 8.8$ Hz, CH), 7.74 (dd, 1 H, $J = 8$, 8 Hz, CH), 7.59 (d, 2 H, $J = 9$ Hz, CH), 7.63 (m, 2 H), 7.52 (d, 1 H, $J = 8.8$ Hz, CH), 7.36 (br d, 1 H, $J = 1.5$ Hz, CH), 6.50 (br s, 2 H, $CH_2$), 4.56 (Br s, 1 H, $CH_2$), 4.88 (dd, 1 H, $J = 9.7$, 11 Hz, $CH_2$), 4.19 (br s, 1 H, $CH_2$), 3.54 (br s, 4 H), 2.90 (br s, 3 H, $CH_3$), 2.73 (s, 3 H, $CH_3$), 1.50 (s, 9 H, $C(CH_3)_3$); LC-MS (ESI) $m/z$ $[M+H]^+$ calcd for $C_{40}H_{40}N_6O_6$: 701.3, found: 701.48; HRMS (ESI) $m/z$ $[M+H]^+$ calcd for $C_{40}H_{40}N_6O_6$: 701.3082, found: 701.3065.

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samples of the (R)-enantiomer of adduct **1b** prepared from incubations of the (R,S)-enantiomer of **7** with calf thymus DNA.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2018.09.057.

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