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Amino DSA analogues as payloads for antibody-drug conjugates with multiple sites for conjugation. Initial studies and solid phase synthesis ☆

Marco M.M.D. Cominetti, Zoë R. Goddard, Chloe E. Howman [1], Maria A. O'Connell, Mark Searcey *

School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

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ABSTRACT

Duocarmycins are highly potent and promising anticancer payloads for ADC applications. They tolerate a range of chemical modifications which allow the chemist to modulate both their biophysical and pharmacological properties. The possibility to synthesize these payloads on resin and orthogonally add linkers while immobilized on the solid phase, would allow a combinatorial design of payload analogues with linkers, potentially aided by automation. Working towards this goal, we report a concise and high yielding synthesis of an alkylating unit suitable for solid phase synthesis (**10**, 9 steps, 34% yield) and demonstrate its applicability to the synthesis of duocarmycin SA analogues (**19**, **20**). An intermediate for traditional solution phase synthesis (**8**) is also described in 7 steps and 44% yield. A side reaction with potential application to the stereoselective synthesis of these derivatives has also been described.

The recent clinical success of antibody-drug conjugates (ADCs) for the treatment of cancer has led to an increased interest in highly potent antitumour agents that can act as payloads. Although not limited to these, to date payloads broadly fall into two categories of compounds: microtubule disruptors and DNA damaging agents [1]. In the latter category, the family of compounds known as the duocarmycins and represented by CC-1065, duocarmycin SA and yatakemycin, are amongst the most well studied of agents (Fig. 1) [2–5]. They exert extremely potent antitumour activity through their ability to alkylate the N-3 of adenine in the minor grove, undergoing a binding induced conformational change that activates them upon interaction with the DNA [6].

Key to the activity of this class of compounds is the spirocyclopropylcyclohexadienone group and, in spite of the apparent stability of the duocarmycins in solution in the absence of DNA, much effort has been expended on blocking the cyclisation of the *seco*-form of the compound in order to release it and convert it to the active form at the appropriate time and in the appropriate place for the molecule to be useful as a drug. The most clinically advanced ADC containing a duocarmycin that is currently under phase III clinical trial, trastuzumab duocarmazine (also known as SYD985) contains an alkylating subunit that is attached to the ADC through the phenol group of the ring opened *seco*-form [7,8].

An alternative approach to duocarmycin analogues is through the development of amino *seco*-DSA. Tercel and co-workers, over several years, have demonstrated that replacing the phenol functionality of *seco*-alkylation subunits with an aniline functionality can generate compounds with similar potent antitumour activity [9–11]. This presumably occurs through an imine intermediate, which could be isolated for an amino CBI derivative [12].

The introduction of an amino group into the DSA alkylating group core structure is intriguing from the point of view of designing ADCs, as it offers a different chemical reactivity for conjugation, for the design of prodrugs or for the reactivity of the alkylating unit.

We have described the solid phase synthesis (SPS) of duocarmycin analogues that incorporate the DSA alkylation subunit and have applied this to the rapid and efficient synthesis of small molecule-drug conjugates and peptide drug conjugates [13–15]. The incorporation of amino *seco*-DSA into a solid phase synthesis approach offers several advantages over the parent hydroxy- substituted structure. During SPS, the most convenient protecting group for the DSA phenol is a benzyl group but as the length of the peptide that incorporates the DSA increases, removal of the benzyl group becomes more problematic. Incorporation of other protecting groups to replace the benzyl group leads to cumbersome protecting group manipulations in the subunit synthesis. With amino *seco*-DSA, the protection for the amine can potentially be a nitro-group, for which on-resin reductions are known. Finally,

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* Corresponding author.
E-mail address: m.searcey@uea.ac.uk (M. Searcey).
[1] Current Address: Department of Chemistry, University of York, Heslington, York YO10 5DD, UK

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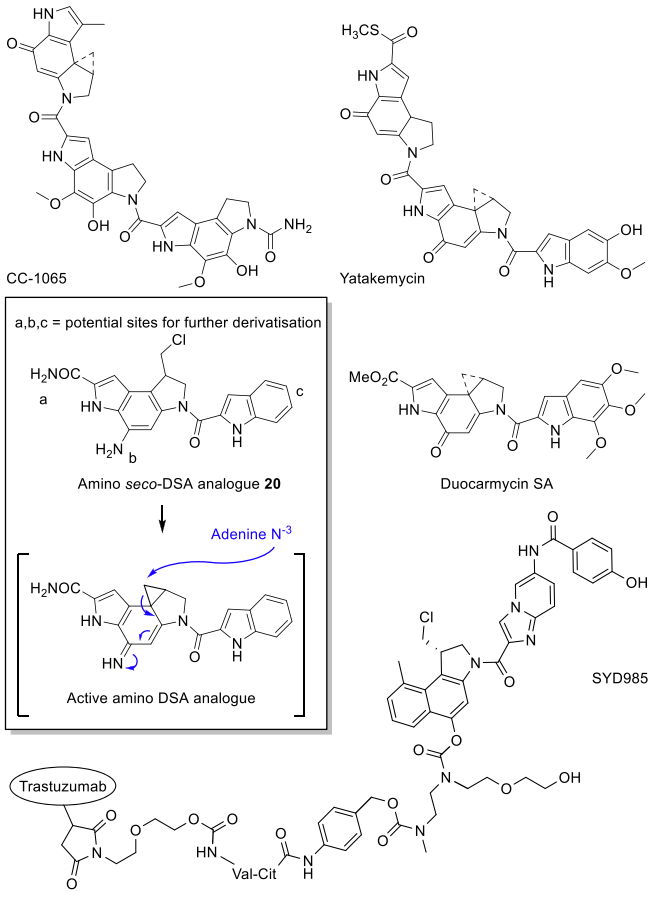
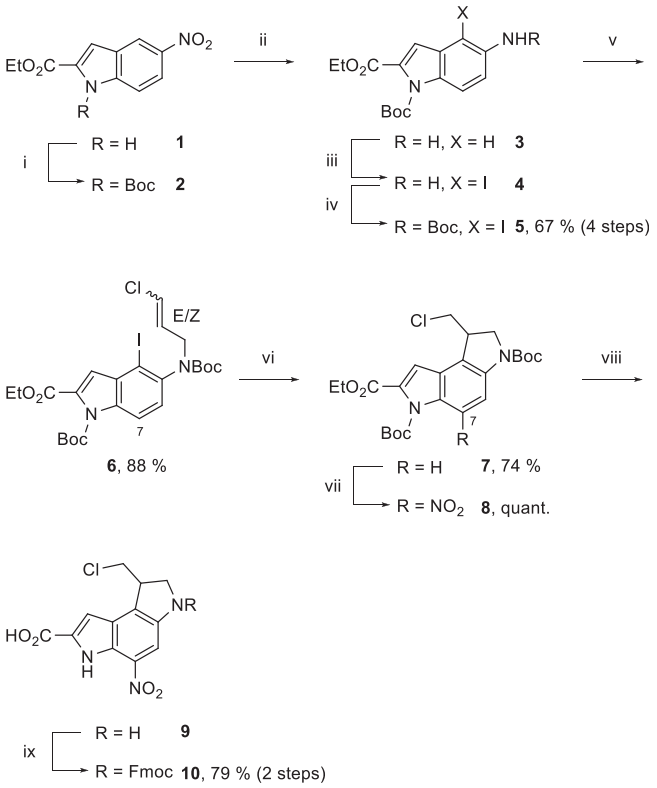


Fig. 1. Examples of the duocarmycin family of compounds and Phase III clinical candidate SYD985. The amino *seco*-DSA analogue described in this work and the alkylation mechanism are shown in the rectangle.



Scheme 1. Synthesis of compound **10.** i) Boc_2O, DMAP, CH_2Cl_2, RT; ii) NH_4Cl, Zn dust, THF, H_2O, RT; iii) NIS, DMF, 0 °C; iv) Boc_2O, TEA, dioxane, reflux; v) NaH 60% in mineral oil, anhydrous DMF, N_2, 0 °C, 1,3-dichloropropene, RT; vi) AIBN, TTMSS, degassed anhydrous toluene, N_2, 90 °C; vii) fuming HNO_3, nitromethane, 0 °C to RT; viii) BBr_3, anhydrous CH_2Cl_2, N_2, RT; ix) α-pinene, FmocCl, dioxane, N_2. Total yield 34% over 9 steps.

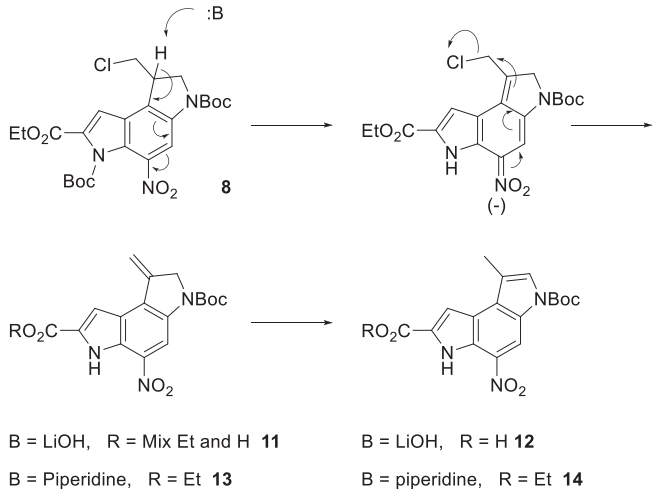
the amino group offers different reactivity but also, during the synthesis, it is possible to allow for different sites of conjugation on the peptide – either through the amino DSA, the growing peptide chain, through the side chains of incorporated amino acids or, following cleavage, through the C terminus of the peptide.

In this paper, we describe initial work towards the development of such flexible systems for ADC payload development, with the synthesis of an Fmoc-protected nitro-DSA derivative and its incorporation into a solid phase method to generate *seco*-amino DSA-indole **20**.

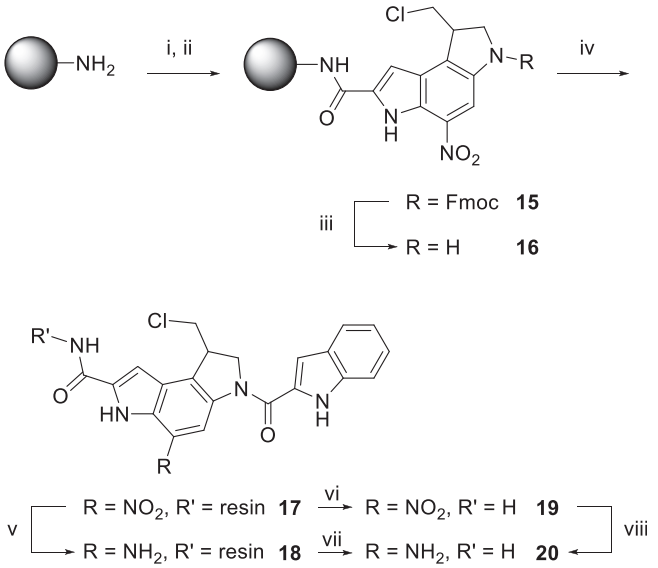
Commercially available ethyl 5-nitroindole-2-carboxylate **1** was initially Boc-protected on the indole nitrogen. A subsequent reduction with zinc and ammonium chloride led to the amino derivative **3**. Iodination to **4** with *N*-iodosuccinimide is regioselective for position 4 without acid catalysis, which otherwise leads to reduced regioselectivity. Installation of a Boc group on the amine in position 5 was achieved by heating at reflux with an excess of Boc dicarbonate and TEA in 1,4-dioxane, and leads exclusively to the mono-protected compound **5**. Despite the apparent purity of the product, purification by flash chromatography was essential to obtain an efficient alkylation in the following step. After purification, we obtained an excellent yield of 67% over four steps (Scheme 1).

Deprotonation with sodium hydride and subsequent alkylation with dichloropropene led to compound **6**, which undergoes an efficient 6-*exo*-*trig* radical cyclisation to **7** initiated by 2,2′-azobis(2-methylpropionitrile) (AIBN) in the presence of tris(trimethylsilyl) silane (TTMSS) as a reducing agent. The nitration of a similar amino *seco*-CPI has been reported by Tercel's group as poorly regioselective [11]. To our surprise, careful control of the amount of nitric acid employed, led to **8** with exclusive regioselectivity for position 7, to the point that purification at this stage was not required and the product could be obtained in quantitative yield after extractive work-up. A slight increase of nitric acid from 1.5 to 2 equivalents led to loss of the observed selectivity. The reason for the difference in selectivity we observed, compared to the one previously reported, is likely due to the additional Boc protection at the indole nitrogen and the chloromethyl group instead of hydroxymethyl and its protected analogues. To obtain **10**, we initially explored a two-step deprotection of the ethyl ester and Boc groups, as previously described for our O-benzylated DSA-Fmoc derivative [13]. Hydrolysis of the ethyl ester with lithium hydroxide could not be achieved due to rapid deprotonation of the benzylic carbon and subsequent elimination of chloride. This side reaction led to an intermediate alkene (**11**, Scheme 2), as previously observed in nitro *seco*-CI [16] and postulated for *seco*-CBI analogues [17]. Under these conditions, the alkene could not be isolated, as it rapidly isomerises to its thermodynamically favoured aromatic isomer **12.**

Simultaneous hydrolysis of both Boc and ethyl ester groups was attempted under acidic conditions, but the results were not encouraging, with either incomplete reactions or decomposition. Therefore, we looked at neutral cleavage conditions and satisfactorily, boron tribromide in dichloromethane produced complete deprotection at room temperature, giving an unexpectedly clean crude **9**, which was immediately used in the following step. Fmoc protection proved to be difficult to control. Use of DIPEA and one equivalent of Fmoc-Cl in THF led to incomplete conversion and attempts to control the quantity of base and Fmoc-Cl were unsuccessful. Replacing DIPEA and THF with α-pinene, to remove HCl,



Scheme 2. Base induced elimination of chloride and aromatization of the indoline ring. The Boc group on the initial indole nitrogen is not stable under basic conditions. The ethyl ester is hydrolysed only in the presence of LiOH.



Scheme 3. Solid phase synthesis of amino *seco*-DSA analogues **19** and **20**. i) **10**, HATU, DIPEA, DMF, RT; ii) AcCl, DIPEA DMF, RT; iii) 20% piperidine, DMF, RT; iv) indole-2-carboxylic acid, EDCI, pTSA, DMA, RT; v) $SnCl_2{\cdot}2H_2O$, DIPEA, NMP, RT; vi and vii) 45% TFA, 45% CH_2Cl_2, 5% TIPS, 5% H_2O; viii) PtO_2, H_2, THF, RT.

and dioxane, led to **10** in good yield (79% over two steps). The synthesis of the orthogonally protected HOOC-DSA(NO_2)-Fmoc (**10**) was thus obtained over nine steps with a total yield of 34% (See Scheme 1).

With a suitably protected alkylation subunit in hand, duocarmycin DSA analogues with an alkylating unit and a simplified binding unit were synthesised to demonstrate its applicability (See Scheme 3). As previously shown [13], the acid at the C-terminus would reduce the biological activity of the product, therefore, we used Novapeg Rink amide, which has excellent swelling properties and would reveal a primary amide end group upon cleavage. Compound **10** was coupled to the resin with HATU and DIPEA, and the resin was subsequently capped to avoid truncated sequences.

Fmoc deprotection was carefully controlled. Extended treatment with high concentrations of piperidine in DMF leads to elimination of HCl, (**13**) as described for LiOH. The milder base allowed isolation of the alkene, although additional treatment or aging, leads to the aromatic isomer **14**. The reaction was investigated in solution (see ESI) and after 20 min, in the presence of 20% piperidine in DMF, a 6% conversion was obtained. On resin, complete deprotection of the Fmoc group could be ensured after three, 4 min treatments, with negligible by product formation (assessed by HPLC, data not shown).

Due to the presence of the deactivating nitro group, the coupling of the following unit is far more demanding than reported for the *seco*-hydroxyl-DSA version [13]. Coupling was not successful with commonly used reagents: HATU/DIPEA, HBTU/DIPEA and DIC/ethyl cyano(hydroxyimino)acetate (Oxyma Pure). Therefore, we opted for an acid catalysed method with EDCI and anhydrous *p*-toluenesulfonic acid (pTSA) in dimethylacetamide (DMA), adapted from previous work by Tercel and co-workers [17]. Although repeating the reaction twice, with 8 equivalents of indole each was required, full conversion could be obtained. Cleavage with a TFA/CH_2Cl_2/TIPS/H_2O-45/45/5/5 mixture led to **19**, which was obtained in 88% yield, based on resin substitution, and 29% yield in respect to compound **10**. It is worth noting that we repeated the coupling of **10** twice, to maximise resin substitution, at the expense of the yield calculated against **10**. For the synthesis of **20**, the nitro group was reduced by treatment with a 2 M solution of $SnCl_2{\cdot}2H_2O$ in NMP in the presence of fifteen molar equivalents of DIPEA. Although issues have been reported with unreliable reduction of nitro groups using tin chloride [18,19], in our hands this approach gave consistent and reproducible results, and did not require heating. However, it has to be noted that the choice of the solvent is critical: DMF and DMF-CH_2Cl_2 mixtures gave non-reproducible results, and seemingly random precipitation of salts, independent of the presence of base and/or resin. The product was subsequently cleaved from the resin with the same cocktail used for **19**. The presence of water and methanol during the cleavage and the purification steps raised a question about the stability of amino compound **20**, which may cyclise and reopen upon nucleophilic attack by water or methanol, converting to the alcohol or the methyl ether derivatives, respectively. It was interesting to note that we did not isolate either product, and the presence of the chlorine atom was clear, matching both the mass and isotopic pattern in high resolution mass spectrometry. This result suggests that under these conditions the aniline ring is not able to undergo spirocyclisation at a significant rate. Protonation of the aniline by TFA and subsequent impairment of its electron donating effect is probably a major reason, and in fact, **20** was isolated as a TFA salt following purification. Compound **20** was obtained in 71% yield, based on resin substitution, and 24% yield in respect to compound **10**. For comparison, **20** was also obtained by reduction of **19** using H_2 in the presence of PtO_2 as catalyst. While high resolution mass spectrometry analysis indicated that the compounds were identical, NMR analysis confirmed that **20** was obtained as a TFA salt using the on resin synthesis and following purification.

We described a convenient large scale synthesis of an alkylating unit based on a nitro *seco*-DSA unit with suitable protecting groups for its application to solid phase synthesis. The unit could be obtained in only nine steps and a total yield of 34%, from a 25 g starting material batch. It is worth noting that compound **8**, which was obtained with an impressive 44% yield, could be used for the synthesis of nitro *seco*-DSA derivatives in solution, as previously described by Tercel's group, effectively reducing the number of steps required to synthesise their equivalent unit from twelve steps to seven (however, we note that our ester bears an ethyl group instead of a methyl) [11]. Description of the side reactions leading to **11** and **13**, which may appear as a hindrance, is worth further exploration, as it paves the way for a potential stereoselective hydroboration, [20] which would allow direct access to individual enantiomers. Finally, we demonstrated the use of our unit

in solid phase synthesis to obtain both a nitro and an amino *seco*-DSA derivative. This work lays the foundation for the development of amino-yatakemycins and the synthesis of drug-linker units *via* orthogonal solid phase synthesis, which will be reported in following publications, together with the biological activity and alkylating properties of the derivatives described herein.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.153058.

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