

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

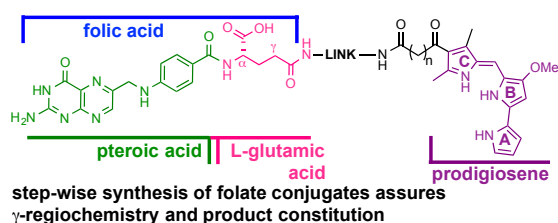
Carlotta Figliola,¹ Estelle Marchal,¹ Brandon R. Groves and Alison Thompson*

Department of Chemistry, Dalhousie University, PO BOX 15000, Halifax, NS, B3H 4R2, Canada

¹Equal contributions

*Alison.Thompson@dal.ca; (T) 1-902-494-3305

Table of Contents Graphic



Abstract

Despite the vast literature that describes reacting folic acid with a pharmacophore, this route is ineffective in providing the correct regioisomer of the resulting conjugate. We herein present a step-wise route to the preparation of nine folate conjugates of the tripyrrolic prodigiosene skeleton. The strict requirement for step-wise construction of the folate core is demonstrated, so as to achieve conjugation at only the desired γ -carboxylic acid and thus maintain the α -carboxylic site for folate receptor (FR α) recognition. Linkages via ethylenediamine, polyethylene glycol and glutathione are demonstrated.

Introduction

Prodigiosin is a tripyrrolic, red pigmented natural product, produced by Gram positive and Gram negative bacteria, including certain strains of *Serratia mercrescens* (Figure 1).^{1,2} Prodigiosin is well known to exhibit immunosuppressive, antimicrobial and anticancer properties, yet modern uses extend from sunscreen³ to antibacterial dyes for silk⁴ and cotton.⁵ Known mechanisms of

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

bioactivity including H^+/Cl^- exchange, oxidative DNA cleavage through Cu(II) chelation and signal-transduction interference.⁶⁻¹⁵ Synthetic mimics of prodigiosin, named prodigiosenes,¹⁶ with modifications on the A-, B- or C-ring¹⁷⁻²⁴ have been shown to maintain the biological activity of the parent compound yet with 100-fold improvements in selectivity towards malignant cells and with 100-fold reduction of systemic toxicity in mice compared to the natural product ($R = CO_2iPr$, $R^1 = Me$, Figure 1).²⁵ Furthermore, the study of these synthetic analogues has enriched the knowledge of structure-activity relationships (SAR) for a better understanding of biological activity of prodigiosin.

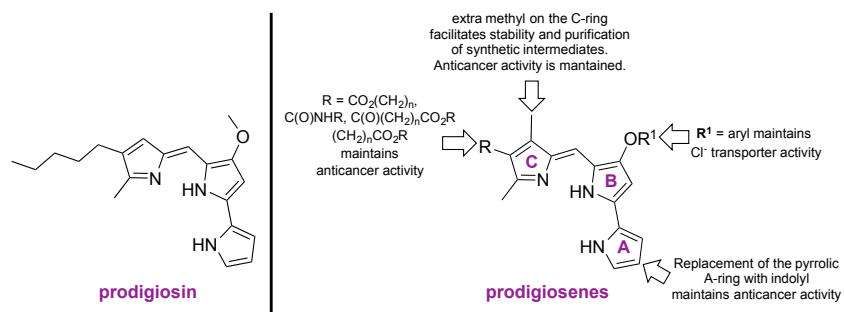


Figure 1. Naturally occurring prodigiosin, and SAR of prodigiosene analogues.¹⁷⁻²⁵

Folic acid (vitamin B9, Figure 2) is considered²⁶ a promising biomarker for triple-negative breast cancer (TNBC).²⁷ More than 50% of TNBCs overexpress folate receptor alpha (FR α), as do a limited number of other cancers²⁸ (*e.g.* ovarian cancers)^{29, 30} along with those tissues where TNBC is likely to result in metastasis, such as brain and lung.³¹ Importantly, FR α is present on only a few types of healthy cells (activated macrophages and proximal tubules of kidneys),³² and, crucially, the uptake of the vitamin folic acid (as a folate salt) is not mediated by FR α .³³⁻³⁵ Consequently, cognizant that folate-conjugated drugs do not generally enter normal cells,³⁶ folic acid has been employed in the synthesis of multiple drug-delivery systems for chemotherapeutic treatment and imaging.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

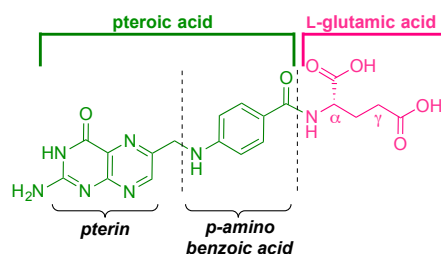


Figure 2. Folic acid, or vitamin B9.

Given the potency of prodigiosenes and the targeting ability of folate, we herein report the synthesis of the first series of folate-prodigiosene conjugates. In doing so, we emphasize the ineffectiveness of the much-utilised direct synthetic approach to couple unmodified folic acid with the desired pharmacophore. Our observations and comments contrast with the vast literature that apparently describes the folate-targeted bioactivity of unpurified and uncharacterised material that results from such direct coupling. We categorically demonstrate that this direct approach, despite its wide use by others, gives rise to mixtures that include both regioisomers featuring the pharmacophore coupled to both carboxyl sites of folic acid. Instead, we demonstrate the requirement for step-wise construction of the folate core, so as to achieve conjugation at only the desired γ -carboxylic acid and thus maintenance of the α -carboxylic site for FR α recognition.³⁷

Results and discussion

Our folate-prodigiosene conjugates (Figure 3) feature prodigiosene **1**³⁸ as the pharmacophore, with varied aliphatic spacer length ($n = 2, 4, 8$) leading to the folate moiety. The conjugated carbonyl group on the β -position of the C-ring has been shown to facilitate purification of prodigiosene intermediates²¹ and so it, and the adjacent β -methyl substituent, were incorporated into the synthetic strategy. Linkers were chosen to increase either the lipophilicity³⁹

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

(ethylenediamine, **DA**) or the water-solubility and biocompatibility^{40, 41} (polyethylene glycol, **PEG**) of the conjugates, or to enable intracellular redox-mediated cleavage by glutathione (disulfide, **SS**, Figure 3).⁴²⁻⁴⁴

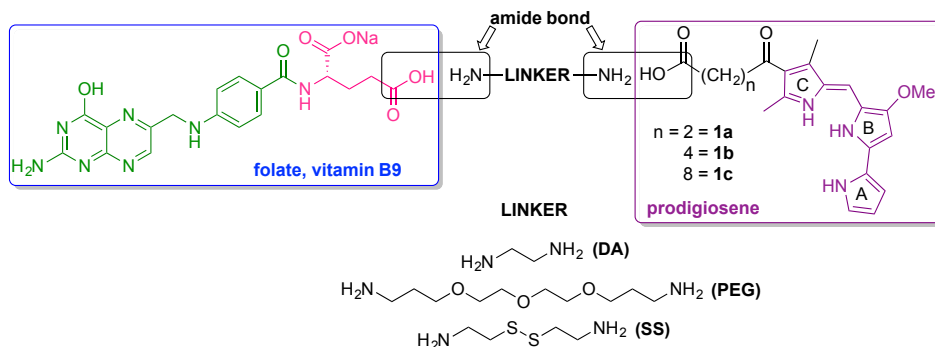


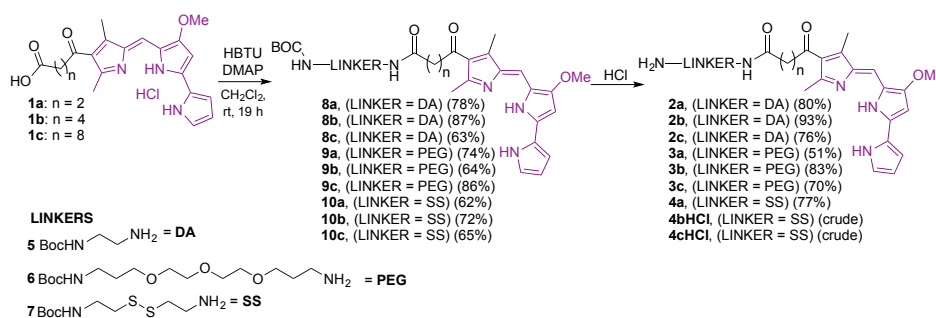
Figure 3. Approach to targeted folate-prodigiosene conjugates.

Synthesis of linker-prodigiosene conjugates

In order to synthesise the linker-prodigiosene conjugates **2-4a-c**, prodigiosenes **1a-c**³⁸ were coupled to the Boc-protected linkers **5-7**^{39, 40, 45} using HBTU in the presence of DMAP. Compounds **8-10** were thus obtained in moderate-to-excellent yields (Scheme 1, General procedure in experimental section). Deprotection, using HCl, afforded the targeted linker-prodigiosene adducts **2-4** after basic work-up to liberate the respective free-bases. However, prodigiosene-linker conjugates **4b•HCl** and **4c•HCl** were used as crude salts, as the S-S bond was found to be highly sensitive to basic work-up conditions and purification using flash chromatography, both of which caused cleavage of the disulfide.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes



Scheme 1. Synthesis of linker-prodigiosene adducts 2-4.

γ -Coupling to folic acid and synthesis of folate-prodigiosene conjugates

Folic acid formally consists of pteric acid linked to L-glutamic acid through a peptide bond (Figure 2).⁴⁶ The crystal structure of human FR α complexed with folic acid reveals a deep binding pocket with the pterate element of folic acid buried inside the receptor.⁴⁷ In contrast, the glutamate moiety of folic acid sits at the pocket entrance. The α -carboxylic acid of the amino-acid is involved in the interaction with the FR α and, thus, in the metabolism and function of folic acid.³⁷ However, conjugates bound at the pendant γ -carboxylic acid, further away from the binding pocket, readily undergo binding to FR α . As such, in order to leave the α -position unmodified, conjugation of pharmacophores to folic acid must be specific to the γ -carboxylic acid in order to maintain ligand binding affinity.³⁷

As shown in Figure 4, three retrosynthetic strategies can be envisaged to achieve the desired folate-prodigiosene conjugates (top). The simplest method, Strategy A, involves the direct coupling of folic acid with the pharmacophore. Following this approach, many literature reports appear to describe the direct activation of folic acid, followed by addition of the desired amine to afford conjugated folates.⁴⁸⁻⁶¹ However, we met Strategy A with considerable scepticism as the presence of two carboxylic acid moieties within folic acid renders two regioisomeric coupling products clearly tangible. Given the nature of those two carboxylic acid

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

groups, we were unable to find any rationale by which to convince ourselves that direct reaction would result in production of the required γ -conjugate. Furthermore, as conjugates to folic acid are often soluble only in DMSO the separation of the two regioisomers represents a considerable challenge. Adding to our skepticism, we found the literature regarding this direct synthetic approach to be incomplete and unreliable, due to either vagueness of purification techniques or lack of characterization such as to give assurance of purity. Indeed, upon close inspection it appears that there are many published reports that simply use the uncharacterised crude precipitate, obtained following the mixing of folic acid, coupling agent and pharmacophore, in studies relating to targeting using folate. Cognisant that coupling with DCC etc. involves ureas and other synthetic intermediates, and that these often persist in crude product mixtures, we remained unconvinced that Strategy A would be fruitful as we required a procedure that gave unequivocal access to the desired γ -conjugate of folic acid bearing prodigiosenes.

Retrosynthetic Strategies B and C (Figure 4) involve the step-wise construction of suitably protected and activated folic acid from pteronic acid and L-glutamic acid, allowing for the selective conjugation of pharmacophore at the desired γ -carboxyl site. Strategy B requires that the γ -activated, α -protected glutamic acid moiety be coupled with the pharmacophore prior to reaction with the pteronate portion of what will become folate.⁶²⁻⁶⁴ Strategy C requires that the γ -activated, α -protected glutamic acid moiety first be coupled with activated pteronate portion to create γ -activated, α -protected folate prior to reaction with the pharmacophore.⁶⁵ Solid-state resin approaches^{66, 67} recognise the virtues of a step-wise approach yet, compared to the lure of Strategy A, both step-wise strategies obviously demand long synthetic sequences and tedious

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

purification processes and have not enjoyed widespread adoption. Indeed, the vast majority of published routes involving folate conjugates steer away from these step-wise approaches.⁶⁸

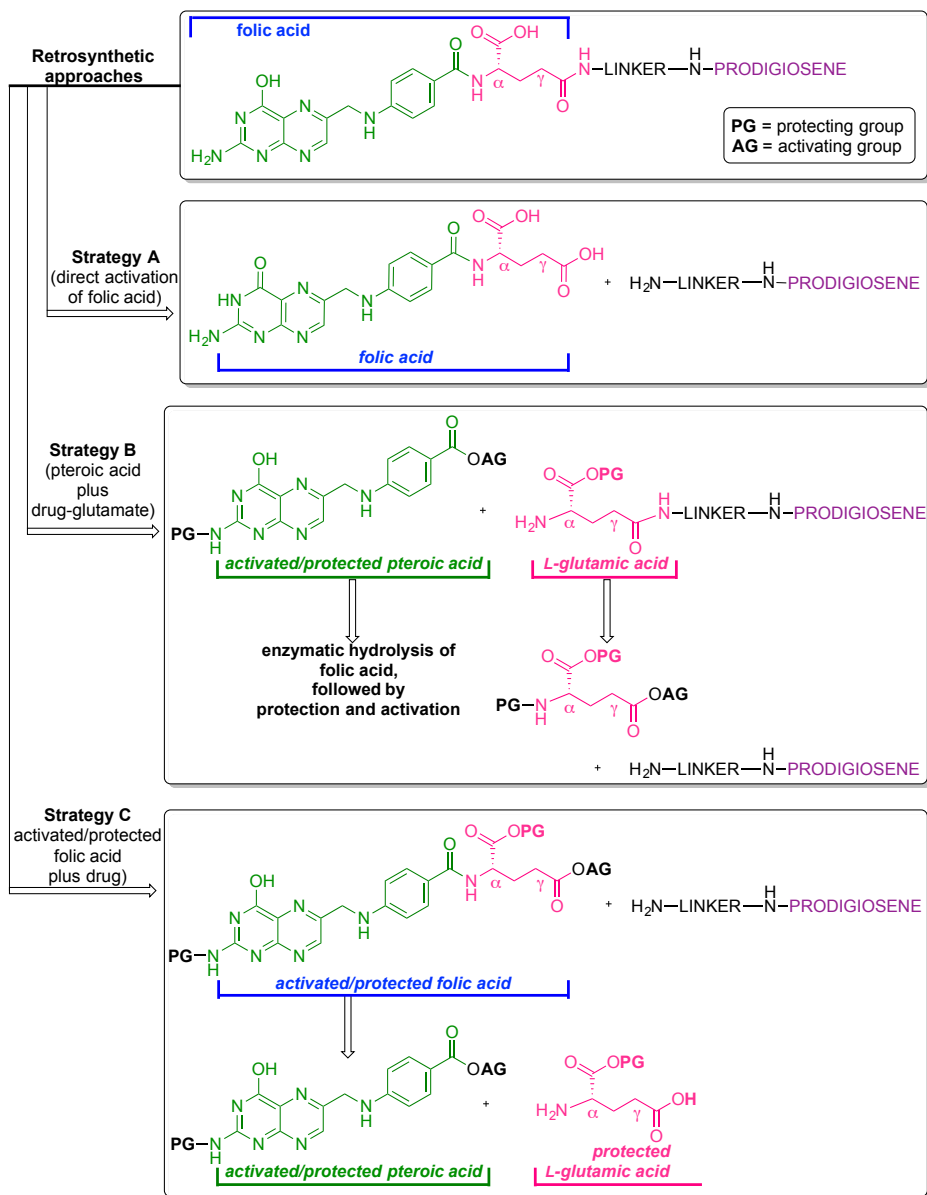


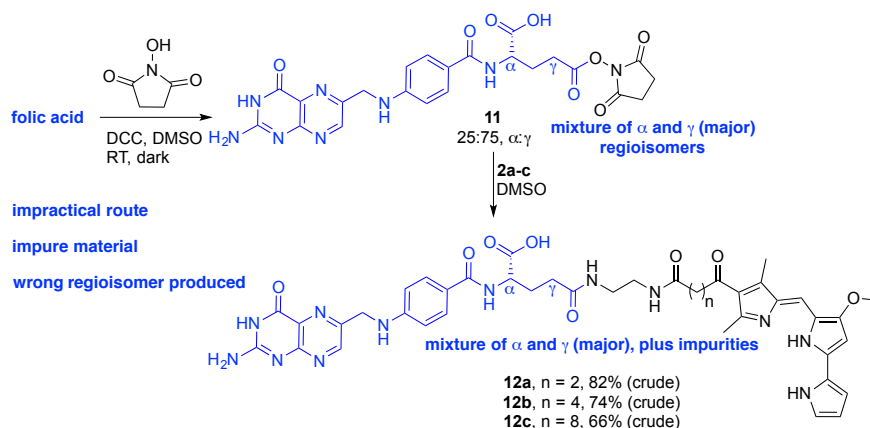
Figure 4. Retrosynthetic approaches for the synthesis of folate-prodigiosene conjugates.

Given the evident popularity of the direct approach offered by Strategy A, we wanted to unequivocally assess the regioselective outcome of reacting folic acid with a coupling agent and an alcohol. We thus treated folic acid with DCC and NHS, and analysed the crude product

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

mixture. Based on the phenyl group signals in the ^1H NMR spectrum (see Electronic Supplemental Information), the crude NHS-adduct was determined to contain α - and γ -adducts in an approx. 25:75 α : γ ratio. A solution of the adduct mixture in DMSO was treated with the linker-prodigiosene conjugate **2a** in an attempt to form the desired amide linkage. Attempts to isolate pure material (**12a**) from the precipitate thus formed were completely unsuccessful (Scheme 2). Rather, the crude mixture was poorly soluble and decomposition occurred on chromatographic media (alumina and silica). Treatment of the NHS-adduct mixture with **2b** and **2c** also resulted in bulk precipitation, and again separation and purification of the highly insoluble material was not possible. Still intrigued by the apparent widespread success of Strategy A, we treated folic acid with DCC, NHS and a simple amine to thus employ another route by which to gain insight regarding the regioselectivity of activation.⁵⁷ A solution of the 25:75 α : γ folate-NHS adduct in DMSO was treated with octyl amine, as a model amine, and the resulting precipitate analyzed using ^1H NMR spectroscopy. Both α - and γ -regioisomers were present, along with the NHS-activated folic acid, and purification proved unsuccessful given the lack of solubility of the material. In short, direct activation following Strategy A was found to be wholly unsatisfactory in the quest towards isolated and pure γ -appended conjugates of folic acid.



Scheme 2. Activation of folic acid with NHS and conjugation to linker-prodigiosenes **2a-c**.

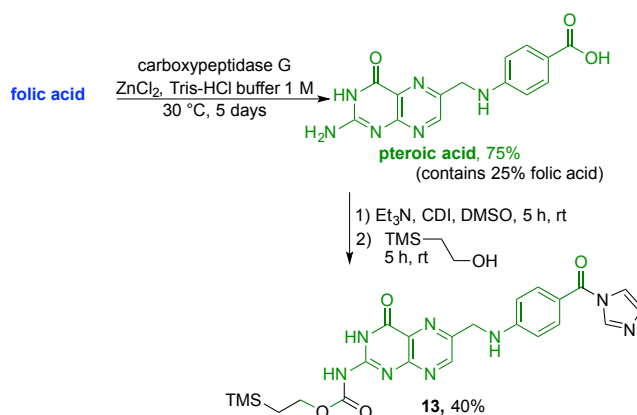
Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

With firm evidence that the direct reaction of folic acid with coupling agent and alcohol/amine does not regioselectively provide the desired γ -regioisomer, we turned our attention to strategies that used sequential protection/activation. We first turned to the applicability of retrosynthetic Strategy B, involving step-wise construction of the folate moiety so as to ensure conjugation at the desired γ -carboxyl site. As shown in Figure 4, the critical step entails coupling of suitably protected (the base portion) and activated (the acid portion) pteronic acid with a derivative of L-glutamic acid that has been pre-linked to the prodigiosene pharmacophore by way of the γ -position. This key coupling would result in the folate-prodigiosene conjugate as a single isomer, courtesy of the previous regiospecific activation of L-glutamic acid.^{62-65, 68, 69} To embark upon Strategy B, pteronic acid was prepared following literature procedure through hydrolysis of folic acid using carboxypeptidase G in the presence of ZnCl₂ at 30 °C.⁶⁵ Carboxypeptidase G was purchased from Sigma and used as is (7 mg; 20 units, since 1 unit = 1 μ mol/min at optimal conditions of 30 °C and pH 7.3). The enzyme operates optimally at pH = 7.3. However, in our hands the reported 0.1 M Tris-HCl buffer was insufficient to maintain stable pH after the addition of folic acid. Therefore, the initial buffer concentration was increased to 1 M and the pH subsequently stabilised in the range of 7.2-7.3 via the addition of solid buffer. After 5 days, ¹H NMR spectroscopic analysis of the crude product mixture revealed 75% conversion of folic acid to pteronic acid. The carboxylic acid of the crude pteronic acid was activated using CDI and the primary amine then protected with a trimethylsilyloxyethyl (Teoc) group, to give **13** after purification using column chromatography on silica (Scheme 3). This purification also allowed the removal of folic acid remaining from the initial step.

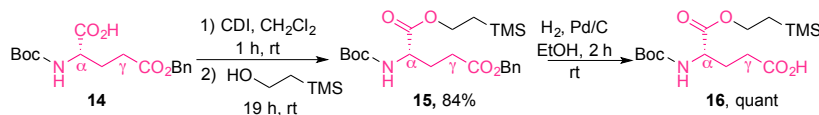
Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes



Scheme 3. Preparation of the activated Teoc-protected pterioic acid **13**.

The L-glutamic acid moiety of folic acid was prepared from its commercially available Boc-L-glutamic acid 5-benzyl ester derivative (**14**), which was first esterified at the α -position using CDI and 2-(trimethylsilyl)ethanol, in order to obtain **15**. Subsequent hydrogenolysis of the benzyl ester at the γ -position gave the protected amino acid **16**, ready for coupling with prodigiosene (Scheme 4).⁶⁵



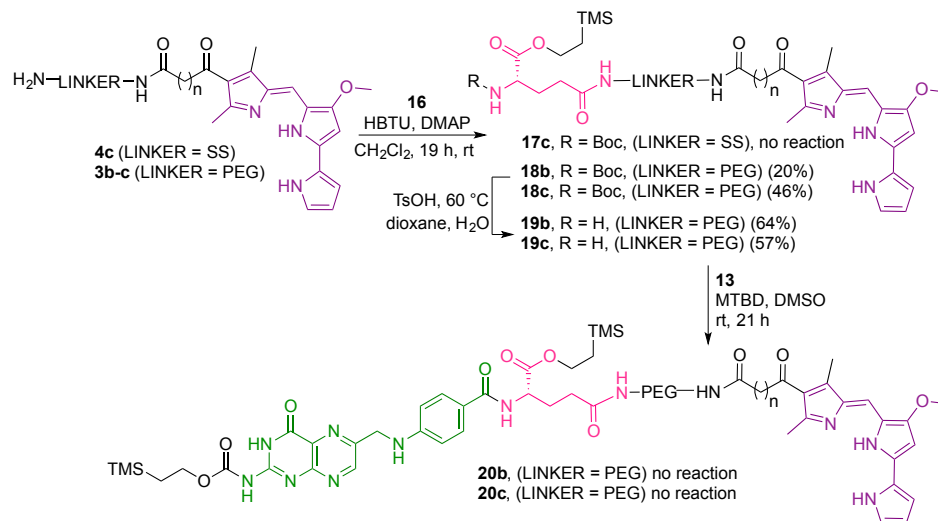
Scheme 4. Preparation of the protected glutamic acid derivative **16**.

Attempts to couple **16** with the dithio linker-prodigiosene conjugate **4c•HCl** in the presence of HBTU and DMAP were unsuccessful and only starting material was recovered (Scheme 5). However, coupling between **3b-c** and the protected amino-acid **16** successfully afforded substrates **18b-c**, thus crucially connecting the prodigiosene pharmacophore with the γ -position of L-glutamic acid, by way of a PEG linker. Boc-deprotection in the presence of TsOH in a mixture of dioxane/water gave the amines **19b-c**. Strategy B (Figure 4) then requires coupling of the drug-appended glutamic acid moiety with the activated pterioic acid, but attempts

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

to couple **19b-c** to **13** resulted in only partial recovery of the substrate prodigiosenes **19b-c** (Scheme 5).



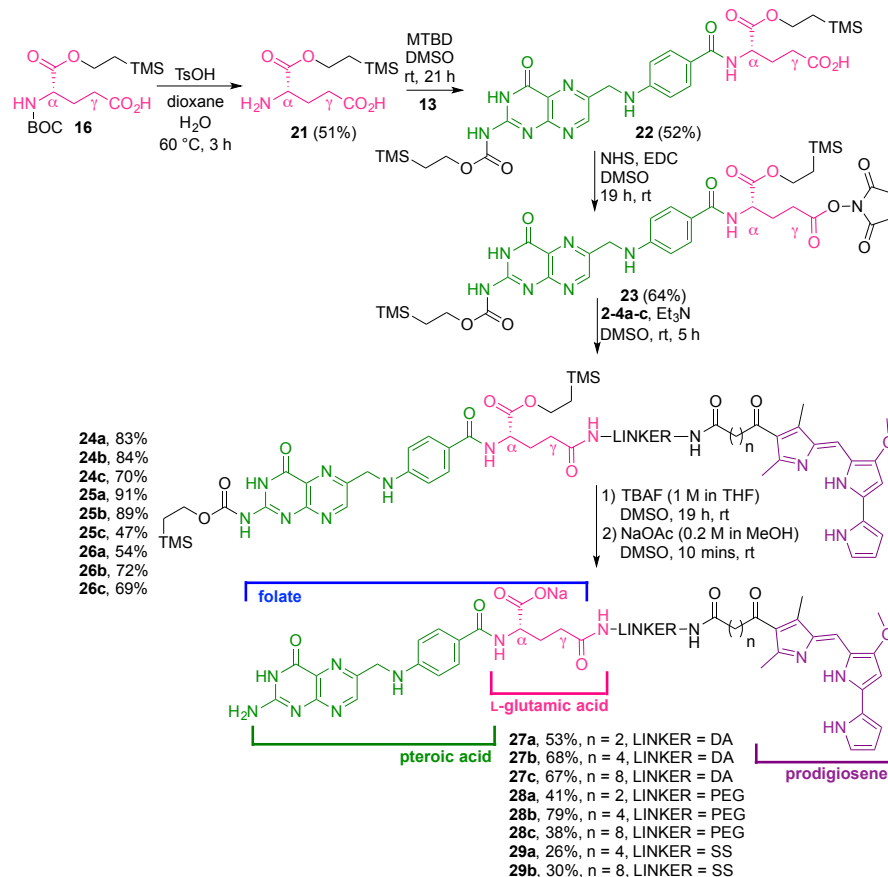
Scheme 5. Attempts to synthesise Teoc-protected folic acid-prodigiosene conjugates **20**.

In turning our attention to Strategy C (Figure 4), the L-glutamic acid derivative **16** was first deprotected at the amino position, and then coupled to the activated pteric acid to successfully afford **22**. This γ -carboxylic acid was then reacted with NHS to give **23** which is activated in the desired position of what will become folate (Scheme 6).⁶⁵ The amines **2-4a-c** were reacted with a solution of **23**, to provide conjugates **24-26a-c**. Subsequently, the Teoc protecting group was removed with TBAF, and the resulting tetrabutylammonium salt precipitated *via* the addition of water. Dissolution of the salt in DMSO, followed by reaction with NaOAc, afforded eight sodium folate-prodigiosene conjugates **27-28a-c** and **29a-b**. However, addition of TBAF to **26c** caused the cleavage of the S-S bond and the corresponding folate-prodigiosene conjugate **29c** could not be isolated (Scheme 6). Clearly the step-wise approach to building folate acid, such that only the γ -carboxyl site is available for coupling, requires more synthetic effort than the direct approach. However, in contrast to Strategy A, the

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

step-wise approaches of Strategies B and C tolerate substantiation of purity and characterization at each synthetic step and should thus be the approaches adopted for the preparation of regioisomerically pure desired conjugates of folate.



Scheme 6. Synthesis of folate-prodigiosene conjugates **27-28a-c** and **29a-b**.

Conclusions

In summary, the first series of folate-prodigiosene conjugates have been synthesised, and full characterization acquired. In order to increase the lipophilicity, the water-solubility and the ease of cleavage, three linkers were chosen to link the folate and the prodigiosene moieties (DA, PEG and SS respectively). Cognizant that the α -carboxylic acid of folic acid is involved in the interaction with FR α , three synthetic approaches were investigated for the preparation of the

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

targeted γ -adduct. The direct and most commonly used literature method involves coupling the unprotected folic acid, activated *in situ*, to the amino-counterpart. Despite the widespread use of this direct approach, often presented along with unsubstantiated claims as to purity and constitution of the product(s) thus obtained, we determined it to be wholly unsatisfactory as it gives mixtures of α - and γ -regioisomers, along with various ureas and other synthetic intermediates presenting as impurities, which are challenging to separate. We evaluated this shortcoming at the activation stage, using simply NHS, as well as through reaction with octyl amine and the prodigiosene pharmacophore. In order to access the required γ -adduct, the hydrolysis of folic acid into its two core components (pteroic acid and L-glutamic acid) was necessary. The subsequent step-wise synthesis of folic acid, with the α -carboxylic acid protected, allowed synthesis of only the desired regioisomer thus, for the first time, connecting folic acid with the prodigiosene pharmacophore. Next steps will involve evaluation of the cytotoxicity of the conjugates, alongside optimization of drug release and internalisation.

Experimental Section

All chemicals were purchased and used as received unless otherwise indicated. Moisture sensitive reactions were performed in oven-dried glassware and under a positive pressure of nitrogen. Air- and moisture-sensitive compounds were introduced via syringe or cannula through a rubber septum. Flash chromatography was performed using either Silicycle ultra-pure silica (230-400 mm) or 150 mesh Brockmann III activated neutral/basic alumina oxide, as indicated. The NMR spectra were recorded using a 500 MHz spectrometer instrument using CDCl_3 , MeOD or DMSO as solvents and are reported in part per million (ppm). Internal solvents were referenced at 7.26 ppm for ^1H and at 77.16 ppm for ^{13}C when using CDCl_3 , at 3.31 ppm for ^1H and at 49.00 ppm for ^{13}C when using MeOD and at 2.50 ppm for ^1H and at 39.5 ppm for ^{13}C

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

when using DMSO- d_6 . Coupling constants (J) are given in Hertz (Hz). Mass spectra were obtained using TOF and LCQ Duo ion trap instruments operating in ESI $^{+/-}$ mode, as indicated. Compounds **1a-c**,²¹ **5-7**,^{39, 40, 45} **11**,⁵⁷ pteronic acid,⁶⁵ **13**,⁶⁵ **15-16**,⁶⁵ and **21-23**⁶⁵ were prepared following literature procedures.

General procedure 1 (GP1): Amines **5-7** (2 eq.), HBTU (1.2 eq.) and DMAP (1.2 eq.) were added to a solution of prodigiosene **1** (1 eq.) in anhydrous CH₂Cl₂ (0.01 M) and the reaction mixture was stirred at room temperature for 16 h under N₂ atmosphere. The solvent was removed under reduced pressure and the crude mixture was partitioned between EtOAc and NaHCO₃ (aq. sat solution). The aqueous layer was extracted with EtOAc (\times 3). The combined organic layers were washed with water (\times 5), brine, dried over Na₂SO₄ and concentrated under reduced pressure.

(Z)-Tert-butyl (2-(4-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-4-oxobutanamido)ethyl)carbamate (8a**)**

Compound **8a** was obtained according to GP1. The crude mixture was purified by column chromatography using A₂O₃ type III basic (CH₂Cl₂:MeOH 100:0, 99:1), followed by A₂O₃ type III neutral (CH₂Cl₂:MeOH, 100:0, 99:1) to afford **8a** (0.098 g, 78%) as dark red glass. ¹H NMR (CDCl₃, 500 MHz) 1.41 (s, 9H), 2.21 (s, 3H), 2.38 (s, 3H), 2.47 (t, J = 6.1 Hz, 2H), 3.02 (t, J = 6.3 Hz, 2H), 3.24 (br s, 2H), 3.31 (br s, 2H), 3.96 (s, 3H), 5.14 (br s, 1H), 6.04 (s, 1H), 6.20-6.21 (m, 1H), 6.35 (br s, 1H), 6.72 (d, J = 3.4 Hz, 1H), 6.76 (s, 1H), 6.89 (s, 1H). ¹³C NMR (CDCl₃, 125 MHz) 12.6, 14.6, 28.5, 29.8, 30.7, 38.3, 40.4, 58.7, 79.5, 96.1, 110.8, 111.9, 113.9, 122.7, 123.3, 126.4, 128.2, 129.8, 141.0, 142.8, 156.7, 160.9, 169.0, 173.7, 196.2. HRMS (TOF) (m/z): [M+H]⁺ calcd for C₂₇H₃₆N₅O₅, 510.2711; found, 510.2719.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

(*Z*)-*Tert*-butyl (2-(6-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-6-oxohexanamido)ethyl)carbamate (8b**)**

Compound **8b** was obtained according to GP1. The crude mixture was purified by column chromatography using Al₂O₃ type III basic (CH₂Cl₂:MeOH 100:0, 99:1), precipitated with CH₂Cl₂:hexanes (1:9), filtered and washed with hexanes to afford **8b** (0.100 g, 87%) as orange glass. ¹H NMR (CD₃OD, 500 MHz) 1.42 (s, 9H), 1.68-1.70 (m, 4H), 2.22-2.26 (m, 2H), 2.39 (s, 3H), 2.66 (s, 3H), 2.81 (t, *J* = 6.6 Hz, 2H), 3.15 (t, *J* = 6.0 Hz, 2H), 3.25 (t, *J* = 6.1 Hz, 2H), 3.92 (s, 3H), 6.08 (s, 1H), 6.26-6.27 (m, 1H), 6.76 (s, 2H), 7.02 (s, 1H). ¹³C NMR (CD₃OD, 125 MHz) 12.3, 15.4, 25.1, 26.7, 28.8, 30.2, 37.0, 40.5, 41.0, 43.1, 59.0, 96.7, 111.2, 111.3, 114.1, 123.6, 123.7, 128.3, 128.8, 129.8, 142.6, 144.3, 162.1, 169.6, 176.3, 200.0 (1 carbon atom unaccounted for). HRMS (TOF) (*m/z*): [M+H]⁺ calcd for C₂₉H₄₀N₅O₅, 538.3024; found, 538.3012.

(*Z*)-*Tert*-butyl (2-(10-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-10-oxodecanamido)ethyl)carbamate (8c**)**

Compound **8c** was obtained according to GP1. The crude mixture was purified by column chromatography using Al₂O₃ type III neutral (EtOAc 100%) followed by Al₂O₃ type III neutral (CH₂Cl₂:MeOH 99:1) and Al₂O₃ type III basic (CH₂Cl₂:MeOH 99.5:0.5, 99:1, 98.5:0.5) to afford **8c** as a red glass (0.116 g, 63%). ¹H NMR (CDCl₃, 300 MHz) 1.26 (s, 8H), 1.42 (s, 9H), 1.58-1.60 (m, 4H), 2.10-2.13 (m, 5H), 2.38 (s, 3H), 2.62 (t, *J* = 7.2 Hz, 2H), 3.24 (br s, 2H), 3.31 (br s, 2H), 3.97 (s, 3H), 5.05 (br s, 1H), 6.06 (s, 1H), 6.18 (s, 1H), 6.25 (br s, 1H), 6.71-6.72 (s, 2H), 6.93 (s, 1H). ¹³C NMR (CDCl₃, 100 MHz) 12.4, 14.0, 24.3, 25.8, 28.5, 29.2, 29.3, 29.4, 36.8, 40.5, 40.7, 42.8, 58.7, 79.7, 96.1, 110.7, 112.1, 113.8, 123.4, 123.6, 126.2, 128.3, 129.7, 132.3,

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

140.5, 142.2, 157.1, 161.1, 169.1, 174.0, 198.3. HRMS (TOF) (m/z): $[M+Na]^+$ calcd for $C_{33}H_{47}N_5NaO_5$, 616.3436; found, 616.3490.

(*Z*)-*Tert*-butyl (18-(2-((4-methoxy-1*H*,1'*H*-[2,2']-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-15,18-dioxo-4,7,10-trioxa-14-azaoctadecyl)carbamate (9a**)**

Compound **9a** was obtained according to GP1. The crude mixture was purified by column chromatography using Al_2O_3 type III basic (EtOAc:hexanes 50:50, 70:30 EtOAc 100:0, CH_2Cl_2 :MeOH 100:0, 99:1) to afford **9a** as an orange glass (0.124 g, 74%). 1H NMR ($CDCl_3$, 500 MHz) 1.42 (s, 9H), 1.73-1.79 (m, 4H), 2.25 (br s, 3H), 2.38 (s, 3H), 2.49 (t, $J = 6.5$ Hz, 2H), 3.02 (t, $J = 6.5$ Hz, 2H), 3.19-3.20 (m, 2H), 3.34 (q, $J = 6.0$ Hz, 2H), 3.51-3.59 (m, 8H), 3.62-3.64 (m, 4H), 3.96 (s, 3H), 5.02 (br s, 1H), 6.02 (s, 1H), 6.22 (br s, 1H), 6.42 (br s, 1H), 6.72 (br s, 1H), 6.78 (br s, 1H), 6.88 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.5, 14.7, 28.6, 29.1, 29.8, 30.5, 37.8, 38.2, 38.6, 58.6, 69.6, 69.9, 70.3 ($\times 2$), 70.6, 79.0, 95.9, 110.8, 111.9, 113.8, 122.8, 123.4, 126.4, 128.2, 129.9, 140.7, 142.6, 156.2, 160.6, 168.8, 172.8, 196.0 (1 carbon atom unaccounted for). HRMS (TOF) (m/z): $[M+Na]^+$ calcd for $C_{35}H_{51}N_5Na_1O_8$, 692.3630; 692.3627.

(*Z*)-*Tert*-butyl (20-(2-((4-methoxy-1*H*,1'*H*-[2,2']-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-15,20-dioxo-4,7,10-trioxa-14-azaicosyl)carbamate (9b**)**

Compound **9b** was obtained according to GP1. The crude mixture was purified by column chromatography using A_2O_3 type III neutral (hexanes:EtOAc 50:50, 40:60, 30:70, 20:80, 10:90, 0:100, then CH_2Cl_2 :MeOH 99:1), followed by twice A_2O_3 type III basic (CH_2Cl_2 :MeOH, 100:0, 99:1) to afford **9b** (0.104 g, 64%) as dark red solid. 1H NMR ($CDCl_3$, 500 MHz) 1.42 (s, 9H), 1.63-1.66 (m, 4H), 1.70-1.80 (m, 4H), 2.14-2.19 (m, 5H), 2.38 (s, 3H), 2.65-2.69 (m, 2H), 3.19 (d, $J = 5.5$ Hz, 2H), 3.34 (dd, $J = 12.0$ and 6.0 Hz, 2H), 3.48-3.62 (m, 12H), 3.97 (s, 3H), 5.02 (m, 1H), 6.04 (s, 1H), 6.19-6.21 (m, 1H), 6.39 (br s, 1H), 6.72 (d, $J = 3.6$ Hz, 1H), 6.76 (s, 1H),

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

6.91 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) 12.3, 13.9, 23.7, 25.4, 28.5, 29.1, 29.7, 36.6, 37.8, 38.5, 42.3, 58.6, 70.0, 70.2 ($\times 2$), 70.6, 78.9, 96.1, 110.6, 111.9, 113.9, 123.3, 123.5, 126.1, 128.1, 129.7, 140.3, 142.3, 156.2, 161.0, 169.1, 172.9, 197.5 (2 carbon atoms unaccounted for). HRMS (TOF) (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{37}\text{H}_{55}\text{N}_5\text{NaO}_5$, 698.4123; found, 698.4102.

(Z)-Tert-butyl (24-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-15,24-dioxo-4,7,10-trioxa-14-azatetracosyl)carbamate (9c)

Compound **9c** was obtained according to GP1. The crude mixture was purified by column chromatography using Al_2O_3 type III basic (EtOAc:hexanes 60:40, 80:20, EtOAc:MeOH 100:0, 99:1) followed by Al_2O_3 type III neutral (EtOAc:hexanes 60:40, 80:20, EtOAc:MeOH 100:0, 99:1, 98:2) and Al_2O_3 type III neutral (EtOAc 100%) to afford **9c** as a red glass (0.200 g, 86%). ^1H NMR (CDCl_3 , 500 MHz) 1.28 (br s, 8H), 1.43 (s, 9H), 1.56-1.63 (m, 4H), 1.75 (quint., $J = 6.0$ Hz, 4H), 2.12 (t, $J = 7.5$ Hz, 2H), 2.20 (s, 3H), 2.39 (s, 3H), 2.63 (t, $J = 7.5$ Hz, 2H), 3.19-3.23 (m, 2H), 3.34 (q, $J = 6.0$ Hz, 2H), 3.49-3.64 (m, 12H), 3.97 (s, 3H), 4.97 (br s, 1H), 6.04 (s, 1H), 6.19-6.21 (m, 2H), 6.72 (d, $J = 3.6$ Hz, 1H), 6.75 (br s, 1H), 6.91 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) 12.4, 14.1, 24.3, 25.9, 28.6, 29.1, 29.3, 29.4, 29.5, 29.8, 36.9, 38.0, 38.6, 42.8, 58.6, 69.7, 70.3 ($\times 2$), 70.6, 70.7, 77.4, 77.6, 79.0, 96.1, 110.7, 112.1, 113.8, 123.3, 126.2, 128.3, 129.6, 140.5, 142.1, 156.2, 160.9, 169.0, 173.2, 198.2 (1 carbon atom unaccounted for). HRMS (TOF) (m/z): $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{41}\text{H}_{63}\text{N}_5\text{Na}_1\text{O}_8$, 776.4569; found, 776.4558.

(Z)-Tert-butyl (2-((2-(4-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-4-oxobutanamido)ethyl)disulfanyl)ethyl)carbamate (10a)

Compound **10a** was obtained according to GP1. The crude mixture was purified by column chromatography using Al_2O_3 type III neutral (EtOAc:hexanes 20:80, 30:70, 40:60, 50:50, 60:40, 70:50, 100:0, EtOAc:MeOH 99:1) to afford **10a** as a red glass (0.052 g, 72%). ^1H NMR (CDCl_3 ,

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

300 MHz) 1.43 (s, 9H), 2.28 (s, 3H), 2.38 (s, 3H), 2.55 (t, $J = 6.4$ Hz, 2H), 2.75-2.82 (m, 4H), 3.04 (t, $J = 6.4$ Hz, 2H), 3.41-3.45 (m, 2H), 3.55 (q, $J = 6.4$ Hz, 2H), 3.97 (s, 3H), 5.05 (br s, 1H), 6.03 (s, 1H), 6.23 (dd, $J = 3.6, 2.4$ Hz, 1H), 6.54 (t, $J = 5.1$ Hz, 1H), 6.23 (dd, $J = 3.6, 2.4$ Hz, 1H), 6.52-6.56 (m, 1H), 6.73 (dd, $J = 3.6, 1.2$ Hz, 1H), 6.81 (br s, 1H), 6.88 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) 12.6, 14.5, 28.5, 30.5, 38.2, 38.4, 39.6, 58.6, 77.4, 79.7, 96.0, 110.8, 111.9, 113.9, 122.7, 123.5, 126.3, 128.1, 130.0, 140.5, 142.8, 156.0, 160.7, 168.9, 173.2, 195.9 (1 carbon atom unaccounted for). HRMS (TOF) (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{29}\text{H}_{40}\text{N}_5\text{O}_5\text{S}_2$, 602.2465; found, 602.2456.

(*Z*)-Tert-butyl (2-((2-(6-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-6-oxohexanamido)ethyl)disulfanyl)ethyl)carbamate (10b)

Compound **10b** was obtained according to GP1. The crude mixture was purified by column chromatography using Al_2O_3 type III neutral (EtOAc:hexanes 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 100:0 then EtOAc:MeOH 99/1) to afford **10b** as a red-brown glass (0.090 g, 75%). ^1H NMR (CDCl_3 , 300 MHz) 1.43 (s, 9H), 1.66-1.68 (m, 4H), 2.21-2.26 (m, 5H), 2.39 (s, 3H), 2.69 (t, $J = 6.3$ Hz, 2H), 2.75 (t, $J = 6.6$ Hz, 2H), 2.82 (t, $J = 6.3$ Hz, 2H), 3.37-3.43 (m, 2H), 3.55 (q, $J = 6.1$ Hz, 2H), 3.98 (s, 3H), 5.02 (br s, 1H), 6.05 (s, 1H), 6.21 (dd, $J = 3.6, 2.4$ Hz, 1H), 6.59 (br s, 1H), 6.73 (dd, $J = 3.6, 1.2$ Hz, 1H), 6.77 (br s, 1H), 6.92 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) 12.4, 14.0, 23.7, 25.3, 28.5, 36.4, 38.0, 38.3, 38.4, 39.6, 42.3, 58.7, 79.7, 96.2, 110.7, 112.0, 114.0, 123.5, 126.2, 128.2, 129.7, 140.5, 142.3, 156.0, 161.1, 169.1, 173.3, 197.6 (1 carbon atom unaccounted for). HRMS (TOF) (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{31}\text{H}_{44}\text{N}_5\text{O}_5\text{S}_2$, 630.2778; found, 630.2770.

(*Z*)-Tert-butyl (2-((2-(10-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-10-oxodecanamido)ethyl)disulfanyl)ethyl)carbamate (10c)

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

Compound **10c** was obtained according to GP1. The crude mixture was purified by column chromatography using Al₂O₃ type III basic (EtOAc:hexanes 50:50, 70:30 EtOAc 100%) followed by Al₂O₃ type III neutral (EtOAc:hexanes 50:50, 60/40) to afford **10c** as a red glass (0.138 g, 65%). ¹H NMR (CDCl₃, 300 MHz) 1.28 (s, 8H), 1.43 (s, 9H), 1.58-1.62 (m, 4H), 2.14 (s, 3H), 2.18 (t, *J* = 7.4 Hz, 2H), 2.39 (s, 3H), 2.62 (t, *J* = 7.4 Hz, 2H), 2.75 (t, *J* = 6.7 Hz, 2H), 2.81 (t, *J* = 5.8 Hz, 2H), 3.39-3.43 (m, 2H), 3.54 (q, *J* = 6.1 Hz, 2H), 3.98 (s, 3H), 5.06 (br s, 1H), 6.06 (s, 1H), 6.18 (t, *J* = 3.0 Hz, 1H), 6.41 (br s, 1H), 6.72 (d, *J* = 3.0 Hz, 2H), 6.93 (s, 1H). ¹³C NMR (CDCl₃, 100 MHz) 12.4, 14.0, 24.3, 25.8, 28.5, 29.3, 29.5, 36.7, 37.9, 38.2, 38.6, 39.7, 42.8, 58.7, 79.7, 96.1, 110.7, 112.1, 113.9, 123.4, 123.6, 126.2, 128.2, 129.8, 140.3, 142.2, 156.1, 161.0, 169.1, 173.7, 198.2 (2 carbon atoms unaccounted for). HRMS (TOF) (*m/z*): [M+H]⁺ calcd for C₃₅H₅₂N₅O₅S₂, 686.3404; found, 686.3386.

(*Z*)-*N*-(2-Aminoethyl)-4-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-4-oxobutanamide (2a**)**

A solution of **8a** (0.038 g, 0.070 mmol) in MeOH (1 mL) was treated with HCl (1.2 mL, 2 M solution in Et₂O, 2.40 mmol) and the reaction mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the crude mixture was dissolved in MeOH (1.5 mL). NaOH (10% aq. solution) was added to the solution until a colour change occurred (dark red to orange). The solvent was removed under reduced pressure and the precipitate was filtered and washed with H₂O to afford **2a** (0.023 g, 80%) as orange solid. M.p.: 119 °C. ¹H NMR (DMSO-*d*₆, 500 MHz) 2.36 (s, 3H), 2.41 (t, *J* = 6.7 Hz, 2H), 2.56 (t, *J* = 6.3 Hz, 2H), 2.69 (s, 3H), 2.99 (t, *J* = 6.8 Hz, 2H), 3.05 (dd, *J* = 12.1, 6.2 Hz, 2H), 3.88 (s, 3H), 6.19 (s, 1H), 6.25 (br s, 1H), 6.70 (s, 1H), 6.79 (br d, 1H, *J* = 3.4 Hz), 7.14 (br s, 1H), 7.78-7.79 (m, 1H), 11.81 (s, 1H). ¹³C NMR (DMSO-*d*₆, 125 MHz) 11.8, 15.4, 29.4, 37.3, 41.4, 42.4, 58.5, 96.3, 110.1 (2C),

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

113.1, 122.1, 122.9, 126.3, 127.5, 128.2, 140.8, 142.1, 159.4, 167.3, 171.6, 195.3 (1 carbon atom unaccounted for). HRMS (TOF) (m/z): $[M+H]^+$ calcd for $C_{22}H_{28}N_5O_3$, 410.2187; found, 410.2177.

(Z)-N-(2-Aminoethyl)-6-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-6-oxohexanamide (2b)

A solution of **8b** (0.100 g, 0.186 mmol) in a mixture of MeOH:CHCl₃ (1:1, 4 mL) was treated with HCl (3.3 mL, 2 M solution in Et₂O, 6.60 mmol) and the reaction mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the crude mixture was dissolved in MeOH (3.8 mL). NaOH (10% aq. solution) was added to the solution until a colour change occurred (dark red to orange). The solvent was removed under reduced pressure and the precipitate was filtered and washed with H₂O to afford **2b** (0.076 g, 93%) as orange solid. ¹H NMR (CD₃OD, 500 MHz) 1.68-1.72 (m, 4H), 2.24-2.27 (m, 2H), 2.40 (s, 3H), 2.66 (s, 3H), 2.79-2.83 (m, 2H), 3.28-3.32 (m, 2H), 3.92 (s, 3H), 6.08 (s, 1H), 6.25-6.27 (m, 1H), 6.76-6.78 (m, 2H), 7.02-7.03 (m, 1H). ¹³C NMR (CD₃OD, 125 MHz) 12.3, 15.4, 25.1, 26.6, 37.0, 41.5, 41.7, 43.1, 59.00, 96.7, 111.2, 111.3, 114.1, 123.5, 123.7, 128.2, 128.9, 129.8, 142.7, 144.2, 162.0, 169.6, 176.6, 199.9. HRMS (TOF) (m/z): $[M+H]^+$ calcd for $C_{24}H_{32}N_5O_3$, 438.2500; found, 438.2488.

(Z)-N-(2-Aminoethyl)-10-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-10-oxodecanamide (2c)

A solution of **8c** (0.073 g, 0.12 mmol) in a mixture of CHCl₃:MeOH (1:1, 6 mL) was treated with HCl 12 N (0.024 mL, 0.29 mmol) and the reaction mixture was stirred at room temperature for 7 h. The solvent was removed under vacuum and NaOH (10% aq. solution) was added drop-wise under stirring until the red solution turned orange-brown. The solvent was removed under

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

vacuum and the precipitate was washed with water to give a dark red solid (0.066 g, 76%). M.p.: 88 °C. ^1H NMR (DMSO- d_6 , 500 MHz) 1.22-1.32 (m, 8H), 1.47-1.49 (m, 2H), 1.56-1.59 (m, 2H), 2.03-2.06 (m, 2H), 2.34 (s, 3H), 2.67 (s, 3H), 2.72-2.74 (m, 2H), 2.99-3.03 (m, 2H), 3.25-3.35 (m, 2H), 3.88 (s, 3H), 6.19 (s, 1H), 6.25 (s, 1H), 6.70 (s, 1H), 6.79 (s, 1H), 7.14 (s, 1H), 7.68 (br s, 1H), 11.81 (br s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) 11.7, 15.3, 23.7, 25.3, 28.6, 28.7, 28.8, 28.9, 35.4, 41.4, 41.8, 42.2, 58.4, 96.2, 110.1 (2C), 113.0, 122.4, 122.9, 126.3, 127.4, 128.2, 140.6, 142.1, 159.3, 167.3, 172.1, 196.6. HRMS (TOF) (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{28}\text{H}_{40}\text{N}_5\text{O}_3$, 494.3126; found, 494.3129.

(*Z*)-*N*-(3-(2-(2-(3-Aminopropoxy)ethoxy)ethoxy)propyl)-4-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-4-oxobutanamide (3a)

A solution of **9a** (0.125 g, 0.18 mmol) in a mixture of MeOH/ CHCl_3 (1:1, 4 mL) was treated with HCl 12 N (0.031 mL, 0.37 mmol) at room temperature. After 30 min additional HCl 12 N (0.030 mL, 0.36 mmol) was added and the reaction mixture stirred for an additional 3 hours. The solvent was removed under reduced pressure and the crude mixture was dissolved in MeOH (2 mL). NaOH (10% aq. solution) was added drop-wise under stirring until the red solution turned orange-brown. The solvent was removed under vacuum and the crude solid was purified using flash chromatography on Al_2O_3 basic type III (EtOAc 100%, CH_2Cl_2 :MeOH 98:2, 95:5, 90:10 then MeOH 100%) to afford **3a** as a dark red glass (0.105 g, 51%). ^1H NMR (CDCl_3 , 500 MHz) 1.68-1.73 (m, 4H), 2.20 (s, 3H), 2.34 (s, 3H), 2.48 (t, $J = 6.5$ Hz, 2H), 2.84 (t, $J = 6.5$ Hz, 2H), 2.97 (t, $J = 6.5$ Hz, 2H), 3.28 (q, $J = 6.5$ Hz, 2H), 3.47-3.60 (m, 12H), 3.95 (s, 3H), 6.03 (s, 1H), 6.18-6.19 (s, 1H), 6.68-6.71 (m, 2H), 6.76 (s, 1H), 6.86 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) 12.5, 14.5, 29.3, 30.4, 30.7, 37.4, 38.0, 40.1, 58.6, 69.3, 69.9, 70.0 ($\times 2$), 70.2, 70.5, 96.0, 110.7,

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

111.7, 113.9, 122.7, 123.5, 126.4, 128.1, 129.8, 140.6, 142.7, 160.7, 168.9, 173.2, 196.1. HRMS (TOF) (m/z): $[M+H]^+$ calcd for $C_{30}H_{44}N_5O_6$, 570.3286; found, 570.3295.

***N*-(3-(2-(2-(3-Aminopropoxy)ethoxy)ethoxy)propyl)-6-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-6-oxohexanamide (3b)**

A solution of **9b** (0.104 g, 0.150 mmol) in a mixture of MeOH:CHCl₃ (1:1, 3.4 mL) was treated with HCl 12 N (0.55 mL, 6.60 mmol) and the reaction mixture was stirred at room temperature for 25 h. The solvent was removed under reduced pressure and the crude mixture was dissolved in MeOH (2 mL). NaOH (6 M aq. solution) was added to the solution until a colour change occurred (dark red to orange). The solvent was removed under reduced pressure and the crude mixture was purified using flash chromatography on Al₂O₃ basic type III (CH₂Cl₂:MeOH, 9:1) to afford **3b** (0.074 g, 83%) as a dark red glass. ¹H NMR (CDCl₃, 500 MHz) 1.63 (br s, 4H), 1.66-1.71 (m, 2H), 1.72-1.77 (m, 2H), 2.15 (s, 3H), 2.17 (br s, 2H), 2.37 (s, 3H), 2.65 (br s, 2H), 2.76 (t, 2H, $J = 6.6$ Hz), 3.30-3.34 (m, 2H), 3.52 (t, 4H, $J = 5.8$ Hz), 3.56 (ddd, 12H, $J = 24.1, 10.1, 8.0$ Hz), 3.96 (s, 3H), 6.04 (s, 1H), 6.18-6.19 (m, 1H), 6.63 (br s, 1H), 6.71 (d, 1H, $J = 3.5$ Hz), 6.74 (br s, 1H), 6.91 (s, 1H). ¹³C NMR (CDCl₃, 125 MHz) 12.4, 14.0, 23.8, 25.5, 29.1, 29.8, 31.0, 33.3, 36.7, 37.7, 39.7, 42.4, 58.6, 69.6, 69.9, 70.2, 70.6, 96.2, 110.7, 112.0, 113.9, 123.4 ($\times 2$), 126.2, 128.2, 129.7, 140.5, 142.3, 161.1, 169.1, 173.0, 197.6. HRMS (TOF) (m/z): $[M+H]^+$ calcd for $C_{32}H_{48}N_5O_6$, 598.3599; found, 598.3577.

***N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-10-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-10-oxodecanamide (3c)**

A solution of **9c** (0.113 g, 0.150 mmol) in a mixture of MeOH:CHCl₃ (1:1, 3.2 mL) was treated with HCl 12 N (0.65 mL of 12 M aqueous solution, 7.80 mmol) and the reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure and the

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

crude mixture was dissolved in MeOH (2 mL). NaOH (2 M aq. solution) was added to the solution until a colour change occurred (dark red to orange). The solvent was removed under reduced pressure and the crude mixture was purified by flash chromatography using Al₂O₃ basic type III (CH₂Cl₂:MeOH, 9:1) to afford **3c** (0.068 g, 70%) as a dark red glass. ¹H NMR (CDCl₃, 500 MHz) 1.24-1.27 (m, 8H), 1.56-1.60 (m, 4H), 1.63 (br s, 4H), 1.65-1.79 (m, 4H), 2.11 (d, 2H, *J* = 7.8 Hz), 2.15 (s, 3H), 2.38 (s, 3H), 2.62 (t, 2H, *J* = 7.4 Hz), 2.77 (t, 2H, *J* = 6.7 Hz), 3.33 (dd, 2H, *J* = 12.2, 6.0 Hz), 3.51-3.63 (m, 12H), 3.97 (s, 3H), 6.05 (s, 1H), 6.17-6.19 (m, 1H), 6.39-6.43 (br m, 1H), 6.70-6.73 (m, 2H), 6.92 (s, 1H). ¹³C NMR (CDCl₃, 125 MHz) 12.4, 14.1, 24.3, 25.9, 29.1, 29.3, 29.4, 29.5, 33.4, 36.9, 37.8, 39.8, 58.6, 69.6 (2C), 70.1, 70.3 (2C), 70.7, 96.1, 110.7, 112.1, 113.8, 123.3, 123.6, 126.2, 128.2, 129.7, 140.4, 142.2, 160.9, 169.1, 173.2, 198.1 (2 carbons uncounted for). HRMS (TOF) (*m/z*): [M+H]⁺ calcd for C₃₆H₅₆N₅O₆, 654.4225; found, 654.4203.

(Z)-N-(2-((2-Aminoethyl)disulfanyl)ethyl)-4-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-4-oxobutanamide (4a)

A solution of **10a** (0.052 g, 0.077 mmol) in a mixture of MeOH:CHCl₃ (1:1, 4 mL) was treated with HCl 12 N (0.024, 0.077 mmol). The reaction was stirred overnight at room temperature and additional HCl 12 N (0.024, 0.077 mmol) was added. The solution was concentrated under reduced pressure and the crude mixture dissolved in MeOH (3 mL). NaOH (10% aq. solution) was added dropwise under stirring until the red solution turned orange-brown. The solvent was removed under vacuum and the crude solid was purified using flash chromatography on Al₂O₃ basic type III (EtOAc 100%, CH₂Cl₂:MeOH, 95:5) to afford **4a** as a dark red glass (0.033 g, 77%), ¹H NMR (CDCl₃, 300 MHz) 2.25 (s, 3H), 2.38 (s, 3H), 2.53 (t, *J* = 6.3 Hz), 2.73-2.80 (m, 4H), 2.97-3.05 (m, 4H), 3.56 (q, *J* = 6.8 Hz, 2H), 3.97 (s, 3H), 6.03 (s, 1H), 6.22 (dd, *J* = 3.8, 2.7

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

Hz, 1H), 6.38 (t, $J = 5.7$ Hz, 1H), 6.73 (dd, $J = 3.8, 1.2$ Hz, 1H), 6.79 (s, 1H), 6.89 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) 12.6, 14.8, 30.6, 38.0, 38.2, 38.5, 40.8, 42.7, 58.7, 96.0, 110.9, 111.9, 113.8, 122.7, 123.2, 126.5, 128.2, 129.8, 142.6, 160.7, 168.9, 173.1, 196.0 (1 carbon atom unaccounted for). HRMS (TOF) (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{32}\text{N}_5\text{O}_3\text{S}_2$, 502.1941; found, 502.1933. HRMS (TOF) (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{32}\text{N}_5\text{O}_3\text{S}_2$, 502.1933; found, 502.1941.

(*S,Z*)-2-(Trimethylsilyl)ethyl 2-((*tert*-butoxycarbonyl)amino)-25-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-20,25-dioxo-9,12,15-trioxa-5,19-diazapentacosan-1-oate (18b**)**

Compound **3b** was obtained following GP1. The crude mixture was purified using Al_2O_3 type III neutral (CH_2Cl_2 100%, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1, 98/2) followed by Al_2O_3 type III basic (CH_2Cl_2 100%, $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99/1) to afford **18b** as a red glass (0.020 g, 20%). ^1H NMR (CDCl_3 , 300 MHz) 0.02 (s, 9H), 0.95-1.01 (m, 2H), 1.42 (s, 9H), 1.65-1.67 (m, 4H), 1.73-1.81 (m, 4H), 1.87-1.95 (m, 1H), 2.08-2.13 (m, 1H), 2.16-2.20 (m, 3H), 2.26 (s, 3H), 2.39 (s, 3H), 2.67-2.69 (m, 2H), 3.31-3.37 (m, 4H), 3.51-3.64 (m, 12H), 3.96 (s, 3H), 4.14-4.20 (m, 3H), 5.44 (d, 1H, $J = 8.1$ Hz), 6.02 (s, 1H), 6.22 (dd, 1H, $J = 3.6, 2.7$ Hz), 6.45 (br s, 1H), 6.59 (br s, 1H), 6.72 (dd, 1H, $J = 3.6, 1.2$ Hz), 6.79 (br s, 1H), 6.89 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) -1.4 (3C), 12.5, 14.7, 17.5, 23.9, 25.5, 28.5 (3C), 28.8, 29.2, 29.8, 32.8, 36.7, 37.8, 37.9, 42.4, 53.5, 58.6, 63.9, 70.0 ($\times 2$), 70.2, 70.6 (4C), 80.0, 96.0, 110.8, 111.9, 113.6, 123.1, 123.3, 126.6, 128.4, 129.3, 141.2, 141.9, 156.0, 160.8, 168.9, 172.1, 172.6, 173.0, 197.6. HRMS (TOF) (m/z): $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{47}\text{H}_{74}\text{N}_6\text{Na}_1\text{O}_{11}\text{Si}_1$, 949.5077; found, 949.5086.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

(*S,Z*)-2-(Trimethylsilyl)ethyl 2-((*tert*-butoxycarbonyl)amino)-29-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-20,29-dioxo-9,12,15-trioxa-5,19-diazanonacosan-1-oate (18c**)**

Compound **3c** was obtained following GP1. The crude mixture was purified using Al₂O₃ type III basic (EtOAc 100%, EtOAc/MeOH 99/1, CH₂Cl₂/MeOH 98/2), followed by Al₂O₃ type III basic (CH₂Cl₂/MeOH 99/1) and Al₂O₃ type III neutral (CH₂Cl₂/MeOH 99/1, 98/2) to afford **18c** as a red glass (0.130 g, 46%). ¹H NMR (CDCl₃, 300 MHz) 0.03 (s, 9H), 0.96-1.02 (m, 2H), 1.28 (s, 8H), 1.43 (s, 9H), 1.59-1.62 (m, 4H), 1.76 (quint., 4H, *J* = 6.0 Hz), 1.87-1.97 (m, 1H), 2.12 (t, 3H, *J* = 7.5 Hz), 2.21-2.26 (m, 5H), 2.39 (s, 3H), 2.64 (t, 2H, *J* = 7.3 Hz), 3.33 (q, 4H, *J* = 6.0 Hz), 3.52-3.64 (m, 12H), 3.96 (s, 3H), 4.16-4.21 (m, 3H), 5.43 (d, 1H, *J* = 8.1 Hz), 6.03 (s, 1H), 6.21-6.23 (m, 2H), 6.56 (br s, 1H), 6.71-6.73 (m, 1H), 6.79 (br s, 1H), 6.91 (s, 1H). ¹³C NMR (CDCl₃, 100 MHz) -1.4 (3C), 12.4, 14.3, 17.5, 24.3, 25.9, 28.4 (3C), 28.8, 29.1, 29.2, 29.3, 29.4, 29.5, 32.8, 36.9, 37.7, 37.9, 42.8, 53.5, 58.6, 63.9, 69.9, 70.1, 70.5 (4C), 77.4, 77.6, 80.0, 96.0, 110.8, 112.1, 113.8, 123.3, 123.5, 126.3, 128.2, 129.7, 142.1, 155.9, 168.9, 172.1, 172.6, 173.4, 198.2. HRMS (TOF) (*m/z*): [M+Na]⁺ calcd for C₅₁H₈₂N₆Na₁O₁₁Si₁, 1005.5703; found, 1005.5713.

(*S,Z*)-2-(Trimethylsilyl)ethyl 2-amino-25-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-20,25-dioxo-9,12,15-trioxa-5,19-diazapentacosan-1-oate (19b**)**

Prodigiosene **18b** (0.020 g, 0.021 mmol) was dissolved in a mixture of dioxane:water (1:1, 1 mL) and PTSA (0.016 g, 0.052 mmol) was added. The reaction was stirred at 60 °C for 3 hours and additional PTSA (0.016 g, 0.052 mmol) was added. After 16h the solvent was removed under reduced pressure the crude solid was purified using Al₂O₃ type III basic (CH₂Cl₂/MeOH

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

99/1) to afford **19b** as an orange glass (0.011 g, 64%). ^1H NMR (CDCl_3 , 300 MHz) 0.04 (s, 9H), 0.96-1.02 (m, 2H), 1.65-1.68 (m, 4H), 1.73-1.82 (m, 5H), 2.04-2.13 (m, 1H), 2.15-2.29 (m, 2H), 2.32-2.40 (m, 2H), 2.68 (s, 3H), 2.72 (s, 3H), 3.30-3.43 (m, 2H), 3.50-3.64 (m, 5H), 3.96 (s, 3H), 4.15-4.21 (m, 2H), 6.01 (s, 1H), 6.23-6.25 (m, 1H), 6.45 (t, 1H, $J = 5.1$ Hz), 6.51 (t, 1H, $J = 4.8$ Hz), 6.72 (d, 1H, $J = 3.6$ Hz), 6.84 (br s, 1H), 6.89 (s, 1H). ^{13}C NMR (CDCl_3 , 100 MHz) -1.4 (3C), 12.5, 14.9, 17.6, 23.9, 25.5, 29.2, 29.3, 30.5, 33.0, 36.7, 37.8, 42.4, 54.2, 58.6, 63.5, 70.0, 70.1, 70.2, 70.6 (4C), 95.9, 110.8, 111.9, 113.5, 123.0, 123.2, 126.7, 128.4, 129.2, 141.4, 141.7, 160.6, 168.8, 172.5, 173.0, 175.9, 197.6.0. HRMS (TOF) (m/z): $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{42}\text{H}_{66}\text{N}_6\text{Na}_1\text{O}_9\text{Si}_1$, 849.4553; found, 849.4557.

(*S,Z*)-2-(Trimethylsilyl)ethyl 2-amino-29-(2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrol-4-yl)-20,29-dioxo-9,12,15-trioxa-5,19-diazanonacosan-1-oate (19c**)**

Prodigiosene **18c** (0.130 g, 0.13 mmol) was dissolved in a mixture of dioxane:water (1:1, 6 mL) and PTSA (0.063 g, 0.32 mmol) was added. The reaction was stirred at 60 °C for 3 hours and additional PTSA (0.063 mg, 0.32 mmol) was added. After 16h the reaction mixture was quenched by addition of NaHCO_3 solid until the dark red solution turned dark brown. The solvent were removed under vacuum and the crude solid was purified using Al_2O_3 type III basic ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 98/2, 95/5) to afford **19c** as an orange glass (0.066 g, 57%). ^1H NMR (CDCl_3 , 300 MHz) 0.03 (s, 9H), 0.96-1.01 (m, 2H), 1.24-1.27 (m, 11H), 1.58-1.60 (m, 4H), 1.74 (quint., 4H, $J = 6.1$ Hz), 2.09-2.14 (m, 3H), 2.19 (s, 3H), 2.29 (t, 2H, $J = 7.3$ Hz), 2.38 (s, 3H), 2.63 (t, 2H, $J = 7.3$ Hz), 3.32 (q, 4H, $J = 6.1$ Hz), 3.38-3.43 (m, 1H), 3.50-3.64 (m, 13H), 3.96 (s, 3H), 4.15-4.21 (m, 2H), 6.04 (s, 1H), 6.19 (dd, 1H, $J = 3.6, 2.7$ Hz), 6.24 (br s, 1H), 6.49 (br s, 1H), 6.71 (dd, 1H, $J = 3.6, 1.1$ Hz), 6.74-6.75 (m, 1H), 6.91 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) -1.4

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

(3C), 12.4, 14.3, 17.6, 24.3, 25.9, 29.2, 29.3, 29.4, 29.5, 29.8, 30.5, 32.9, 36.9, 37.8 ($\times 2$), 42.8, 54.1, 58.6, 63.4, 70.0 ($\times 2$), 70.2, 70.6 (4C), 96.0, 110.7, 112.1, 113.7, 123.2, 123.5, 126.3, 128.3, 129.5, 140.8, 142.0, 160.8, 179.0, 172.4, 173.3, 175.9, 198.2. HRMS (TOF) (m/z): $[M+Na]^+$ calcd for $C_{46}H_{74}N_6Na_1O_9Si_1$, 905.5179; found, 905.5145.

General procedure 2 (GP2): A solution of linker-prodigiosene conjugate (1 eq.) and **23** (1 eq.) in anhydrous DMSO (0.05 M) was treated with NEt_3 (2 eq.). The resulting reaction mixture was stirred at room temperature for 5 h under N_2 atmosphere. Water was added to the reaction mixture until a precipitate formed, which was isolated via microfiltration, then washed 3 times with water and dried under vacuum.

Teoc-FA-DA-prod(C2) (24a)

Compound **24a** was obtained according to GP2 as an orange solid (0.096 g, 83%). M.p.: 148-150 °C. 1H NMR (DMSO- d_6 , 500 MHz) 0.02 (s, 9H), 0.07 (s, 9H), 0.93-0.96 (m, 2H), 1.02-1.06 (m, 2H), 1.37 (s, 6H), 1.92-2.27 (m, 4H), 2.36 (s, 3H), 2.68 (s, 3H), 2.73 (s, 1H), 2.98-3.00 (m, 4H), 3.88 (s, 3H), 4.10-4.16 (m, 2H), 4.25-4.30 (m, 2H), 4.35 (br s, 1H), 4.57 (br s, 2H), 6.17 (s, 1H), 6.25 (s, 1H), 6.67 (br d, 2H, $J = 7.0$ Hz), 6.79 (br s, 1H), 6.83 (br s, 1H), 7.11 (br s, 1H), 7.66 (br d, 3H, $J = 7.2$ Hz), 8.16 (br s, 1H), 8.78 (s, 1H), 11.70 (br s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) -1.5 (6C), 11.9, 15.4, 16.8, 17.1, 26.4, 28.2, 29.5, 31.8, 37.3 (2C), 46.0, 52.3, 58.4, 62.4, 64.1, 77.6, 96.3, 110.1, 111.2 (2C), 113.1, 121.3, 122.1, 122.9, 126.4, 127.5, 128.2, 129.0 (2C), 129.8, 140.9, 142.2, 148.9, 150.7, 151.4, 154.9, 155.3, 155.6, 159.5, 160.9, 166.4, 167.4, 171.6, 171.8, 172.3, 195.3. HRMS (TOF) (m/z): $[M+H]^+$ calcd for $C_{52}H_{69}N_{12}NaO_{10}Si_2$, 1077.4793; found, 1077.4748.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

Teoc-FA-DA-prod(C4) (24b)

Compound **24b** was obtained according to GP2 as orange solid (0.096 g, 84%). M.p.: 138-140 °C. ¹H NMR (DMSO-d₆, 500 MHz) 0.00 (s, 9H), 0.05 (s, 9H), 0.86-0.87 (m, 3H), 0.91-0.94 (m, 2H), 1.02-1.05 (m, 3H), 1.24-1.28 (m, 1H), 1.36 (br s, 3H), 1.54 (br s, 4H), 1.91-2.07 (m, 4H), 2.18 (br s, 2H), 2.34 (s, 3H), 2.67 (s, 3H), 2.72 (br s, 2H), 3.06 (m, 4H), 3.87 (s, 3H), 4.09-4.12 (m, 2H), 4.28-4.29 (m, 2H), 4.58 (br s, 3H), 6.18 (s, 1H), 6.24 (s, 1H), 6.65 (br d, 2H, *J* = 7.0 Hz), 6.68 (s, 1H), 6.78 (br s, 1H), 6.99 (br s, 1H), 7.13 (s, 1H), 7.65 (br d, 2H, *J* = 7.0 Hz), 7.77 (br s, 1H), 7.85 (br s, 1H), 8.28-8.31 (m, 1H), 8.81 (s, 1H), 11.55 (br s, 2H), 11.81 (br s, 1H). ¹³C NMR (DMSO-d₆, 125 MHz) -1.5 (6C), 11.8, 15.4, 16.9, 17.1, 23.4, 25.0, 26.4, 28.2, 31.8, 35.5, 38.3, 38.5, 41.6, 46.0, 52.3, 58.4, 62.4, 64.6, 79.2, 96.2, 110.1, 111.3, 113.0, 121.4, 122.3, 122.9, 126.3, 127.5, 128.2, 128.7, 129.1, 129.9, 140.8, 142.2, 149.1, 149.2, 150.7, 151.9, 154.5, 155.0, 159.4, 159.6, 166.4, 167.4, 171.6, 172.2, 172.3, 196.4. HRMS (TOF) (*m/z*): [M+H]⁺ calcd for C₅₄H₇₃N₁₂O₁₀Si₂, 1105.5106; found, 1105.5086.

Teoc-FA-DA-prod(C4) (24c)

Compound **24c** was obtained according to GP2 as a dark red solid (0.098 g, 70%). M.p.: 124-126 °C. ¹H NMR (CDCl₃, 500 MHz) 0.02 (s, 9H), 0.05 (s, 9H), 0.99-1.05 (m, 4H), 1.21-1.25 (m, 8H), 1.56 (s, 4H), 1.98 (s, 1H), 2.13-2.17 (m, 3H), 2.26-2.34 (m, 8H), 2.57-2.61 (m, 2H), 3.21 (br s, 1H), 3.33 (s, 1H), 3.43-3.50 (m, 2H), 3.94 (s, 3H), 4.20-4.29 (m, 4H), 4.58 (s, 2H), 4.65-4.68 (m, 1H), 5.56 (br s, 1H), 5.97 (s, 1H), 6.25 (br s, 1H), 6.59 (br s, 2H), 6.72 (br s, 1H), 6.84 (s, 2H), 6.96-6.97 (m, 2H), 7.12 (br s, 1H), 7.19 (br s, 1H), 7.63-7.64 (m, 2H), 8.74 (s, 1H). ¹³C NMR (CDCl₃, 125 MHz) -1.5 (6C), 11.8, 15.3, 16.8, 17.1, 23.8, 25.2, 26.3, 28.2, 28.8, 28.9, 31.7, 35.4, 37.8 (2C), 41.8, 46.1, 52.2, 58.4, 62.4, 63.6, 77.6, 96.2, 110.1, 111.2 (2C), 111.3, 113.0, 121.2, 121.3, 122.4, 122.9, 126.3, 127.4, 128.2, 129.0, 129.6 (2C), 140.6, 142.1, 148.5,

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

150.7 ($\times 2$), 152.2, 155.2, 155.6, 159.4, 162.0, 166.3, 167.3, 171.5, 172.3, 196.6. HRMS (TOF) (m/z): $[(M+H)]^+$ calcd for $C_{58}H_{81}N_{12}O_{10}Si_2$, 1161.5732; found, 1161.5700.

Teoc-FA-PEG-prod(C2) (25a)

Compound **25a** was obtained according to GP2 as an orange-brown solid (0.146 g, 91%). M.p.: 114-115 °C. 1H NMR ($CDCl_3$, 500 MHz) 0.01 (s, 9H), 0.06 (s, 9H), 0.96-1.00 (m, 2 H), 1.02-1.06 (m, 2H), 1.20 (t, $J = 7.0$ Hz, 1H), 1.71 (dt, $J = 21.5, 6.0$ Hz, 4H), 2.10-2.14 (m, 2H), 2.19-2.29 (m, 2H), 2.33 (s, 6H), 2.49 (t, $J = 6.7$ Hz, 2H), 2.97 (t, $J = 6.7$ Hz, 2H), 3.23-3.32 (m, 4H), 3.46-3.63 (m, 12H), 3.92 (s, 3H), 4.16-4.19 (m, 2H), 4.28 (t, $J = 7.7$ Hz, 2H), 4.57-4.64 (m, 3H), 5.38 (br s, 1H), 5.93 (s, 1H), 6.26 (s, 1H), 6.56 (d, $J = 7.2$ Hz, 2H), 6.71 (s, 2H), 6.78 (s, 2H), 6.96 (br s, 1H), 7.39 (d, $J = 6.5$ Hz, 1H), 7.67 (d, $J = 7.2$ Hz, 2H), 8.74 (br s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) -1.41 (6C), 12.5, 15.4, 17.5, 17.7, 27.7, 29.0, 29.2, 30.4 (2C), 32.9, 37.6, 37.8, 38.1 (2C), 46.7, 53.1, 58.6, 63.9, 65.6, 65.9 (2C), 69.6, 69.7, 70.0, 70.1, 70.5 (2C), 95.5, 110.8, 111.5, 112.1 (2C), 113.9, 122.5 ($\times 2$), 123.8, 126.5, 127.7, 129.2, 129.8 (2C), 142.6, 149.0, 150.4, 151.5, 154.0, 154.7, 167.5, 168.2, 172.7, 173.1, 196.0 (2 carbons uncounted for). HRMS (TOF) (m/z): $[M+H]^+$ calcd for $C_{60}H_{85}N_{12}O_{13}Si_2$, 1237.5892; found, 1237.5890.

Teoc-FA-PEG-prod(C4) (25b)

Compound **25b** was obtained according to GP2 as a dark red solid (0.101 g, 89%). M.p.: 128-130 °C. 1H NMR ($CDCl_3$, 500 MHz) 0.03 (s, 9H), 0.07 (s, 9H), 0.98-1.01 (m, 2H), 1.04-1.07 (m, 2H), 1.61 (br s, 4H), 1.70-1.72 (m, 2H), 1.75-1.78 (m, 2H), 2.16-2.22 (m, 4H), 2.30-2.39 (m, 8H), 2.61 (br s, 2H), 3.23-3.36 (m, 4H), 3.50-3.60 (m, 12H), 3.94 (s, 3H), 3.97-3.99 (br m, 1H), 4.18-4.22 (m, 2H), 4.27-4.31 (m, 2H), 4.57 (br s, 2H), 4.61-4.66 (m, 2H), 5.44 (br s, 1H), 5.95 (s, 1H), 6.27 (s, 1H), 6.58 (d, 2H, $J = 7.2$ Hz), 6.72-6.74 (m, 2H), 6.81 (s, 1H), 6.90 (br s, 1H), 6.97 (br s, 1H), 7.41 (d, 1H, $J = 7$ Hz), 7.68 (d, 2H, $J = 7.2$ Hz), 8.74 (br s, 1H). ^{13}C NMR ($CDCl_3$,

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

125 MHz) -1.5 (6C), 12.3, 15.1, 17.4, 17.6, 23.7, 25.4, 27.7, 28.5, 29.0, 29.2, 29.7, 32.8, 36.5, 37.5, 37.8, 42.3, 46.7, 53.0, 58.6, 63.8, 65.7, 69.7, 70.0, 70.1, 70.4 (2C), 95.6, 110.8, 111.5, 112.1 (92C), 114.0, 122.5, 123.0, 123.6, 126.4, 127.6, 127.9, 129.1, 129.8 (2C), 139.9, 142.2, 149.1, 150.3, 151.5, 154.1, 154.6, 159.4, 161.9, 167.5, 168.3, 172.6, 172.7, 173.2, 197.4. HRMS (TOF) (m/z): $[(M+H+Na)/2]^{2+}$ calcd for $C_{62}H_{89}N_{12}NaO_{13}Si_2$, 644.3049; found, 644.3030.

Teoc-FA-PEG-prod(C8) (25c)

Compound **25c** was obtained according to GP2. The crude mixture was purified by column chromatography using Al_2O_3 type III neutral (CH_2Cl_2 :MeOH, 9:1) and **25c** was obtained as dark red glass (0.065 g, 47%). 1H NMR ($CDCl_3$, 500 MHz) 0.01 (s, 9H), 0.05 (s, 9H), 0.82-0.89 (m, 1H), 0.96-1.07 (m, 4H), 1.25 (br s, 8H), 1.57-1.58 (br m, 4H), 1.68-1.77 (m, 4H), 2.09-2.14 (m, 2H), 2.19-2.25 (m, 2H), 2.29-2.33 (m, 2H), 2.36 (s, 6H), 2.61 (t, 2H, $J = 7.1$ Hz), 3.27-3.34 (m, 4H), 3.47-3.58 (m, 12H), 3.94 (s, 3H), 4.17-4.22 (m, 2H), 4.26-4.31 (m, 2H), 4.62 (br s, 3H), 5.43 (br s, 1H), 5.98 (s, 1H), 6.24 (br s, 1H), 6.44-6.48 (m, 1H), 6.60 (d, 2H, $J = 8.3$ Hz), 6.72 (br s, 1H), 6.81-6.85 (m, 2H), 6.90 (br s, 1H), 7.40 (d, 1H, $J = 7.2$ Hz), 7.67 (d, 2H, $J = 8.3$ Hz), 8.77 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) -1.4 (6C), 12.4, 15.2, 17.5, 17.7, 24.3, 25.9, 27.9, 29.0, 29.3 ($\times 2$), 29.4, 29.5 ($\times 2$), 29.8 (2C), 32.9, 36.9, 37.7, 38.0 (2C), 42.8, 46.9, 53.1, 58.7, 64.0, 66.1 (2C), 69.9, 70.0, 70.1, 70.2, 70.6, 95.7, 110.9, 111.9, 112.3 (2C), 114.0, 122.8, 123.5, 126.6, 127.9, 129.2, 130.1 (2C), 140.2, 142.2, 149.4, 150.3, 151.8, 154.0, 154.8, 159.6, 161.0, 167.3, 168.5, 172.6, 172.7, 173.5, 198.1. HRMS (TOF) (m/z): $[M+H]^+$ calcd for $C_{66}H_{97}N_{12}O_{13}Si_2$, 1321.6831; found, 1321.6807.

Teoc-FA-SS-prod(C2) (26a)

Compound **26a** was obtained according to GP2 as an orange-brown solid (0.042 g, 54%). M.p.: 149-151 °C. 1H NMR ($CDCl_3$, 500 MHz) 0.00 (1s, 9H), 0.04 (s, 9H), 0.95 (t, $J = 8.5$ Hz, 2H),

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

1.01 (t, $J = 8.5$ Hz, 2H), 1.25 (s, 1H), 2.16-2.20 (m, 2H), 2.26 (s, 6H), 2.33-2.39 (m, 2H), 2.45 (br s, 2H), 2.61 (br s, 2H), 2.71-2.72 (m, 2H), 2.92 (br s, 2H), 3.42-3.51 (m, 4H), 3.91 (s, 3H), 4.15 (t, $J = 8.5$ Hz, 2H), 4.24-4.32 (m, 3H), 4.55 (br s, 2H), 4.65-4.68 (m, 1H), 5.49 (br s, 1H), 5.92 (s, 1H), 6.23 (s, 1H), 6.53 (d, $J = 7.7$ Hz, 2H), 6.69 (s, 1H), 6.72 (s, 1H), 6.91 (s, 1H), 7.06 (br s, 1H), 7.36 (br s, 1H), 7.64 (d, $J = 7.7$ Hz, 2H), 8.71 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) - 1.4 (6C), 12.5, 15.6, 17.5, 17.7, 27.5, 28.5, 29.8, 30.3, 32.8 (2C), 38.0 (2C), 38.7, 38.8, 46.8, 53.1, 58.6, 64.0, 65.9, 95.4, 110.9, 111.3, 112.1, 112.2 (2C), 114.1, 122.4, 124.0, 126.5 (2C), 127.5, 129.2 (2C), 130.0, 142.9, 149.1, 150.4, 151.9, 154.1, 167.6, 168.1, 172.6, 173.2, 173.6, 196.0 (3 carbon atoms unaccounted for). HRMS (TOF) (m/z): $[(M+H+Na)/2]^{2+}$ calcd for $\text{C}_{54}\text{H}_{73}\text{N}_{12}\text{Na}_1\text{O}_{10}\text{S}_2\text{Si}_2$, 596.2211; found, 596.2220.

Teoc-FA-SS-prod(C4) (26b)

A solution of **4b** (0.128 g, 0.203 mmol) in a mixture of MeOH: CHCl_3 (1:1, 4 mL) was treated with HCl 12N (1.2 mL, 14.4 mmol) and the reaction mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure to obtain **4b** as dark purple solid (0.124 g), which was used in the next step without any further purification. HRMS (TOF) (m/z): $[M+H]^+$ calcd for $\text{C}_{26}\text{H}_{36}\text{N}_5\text{O}_3\text{S}_2$, 530.2254; found, 530.2264. Compound **26b** was obtained according to GP2. The crude mixture was purified by column chromatography using Al_2O_3 type III neutral (CH_2Cl_2 :MeOH, 9:1) and **26b** was obtained as an orange solid (0.176 g, 72%). M.p. 135-137 °C. ^1H NMR (CDCl_3 , 500 MHz) 0.02 (s, 9H), 0.06 (s, 9H), 0.81-0.90 (m, 1H), 0.96-1.06 (m, 4H), 1.58 (br s, 4H), 2.09-2.19 (m, 4H), 2.26 (br m, 3H), 2.31 (s, 3H), 2.34-2.40 (m, 2H), 2.57 (br s, 2H), 2.69-2.78 (m, 4H), 3.40-3.53 (m, 4H), 3.93 (s, 3H), 4.16-4.32 (m, 4H), 4.58 (d, 2H, $J = 4.0$ Hz), 4.65-4.72 (m, 1H), 5.45-5.46 (m, 1H), 5.95 (s, 1H), 6.25-6.27 (m, 1H), 6.56 (d, 2H, $J = 8.6$ Hz), 6.72 (br s, 1H), 6.80 (s, 1H), 6.89-6.95 (m, 2H), 7.14 (t, 1H, $J = 5.6$ Hz), 7.32 (d, 1H, $J =$

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

6.7 Hz), 7.65 (d, 2H, $J = 8.6$ Hz), 8.75 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) -1.4 (6C), 12.4, 15.4, 17.5, 17.7, 23.6, 25.3, 27.3, 29.8, 32.9, 36.4, 37.9, 38.1, 38.6, 38.8, 42.2, 46.8, 53.0, 58.7, 64.0, 66.0, 95.6, 111.0, 111.6, 112.2 (2C), 114.2, 122.5, 123.1, 124.0, 126.5, 127.5, 129.2 (2C), 129.9, 139.8, 142.3, 149.2, 149.9, 150.4, 151.8, 154.0, 154.7, 159.3, 161.5, 167.7, 168.3, 172.6, 173.2, 173.8, 197.5. HRMS (TOF) (m/z): $[(M+H+Na)/2]^+$ calcd for $\text{C}_{56}\text{H}_{77}\text{N}_{12}\text{NaO}_{10}\text{S}_2\text{Si}_2$, 610.2376; found, 610.2356.

Teoc-FA-SS-prod(C8) (26c)

A solution of **4c** (0.150 g, 0.22 mmol) in a mixture of MeOH: CHCl_3 (1:1, 4 mL) was treated with HCl 12 N (0.036 mL, 0.44 mmol). After an additional 30 min HCl 12 N (0.030 mL, 0.36 mmol) was added and the reaction mixture stirred for a further 12 hours. The solvent was removed under reduced pressure to afford **4c** as a red glass (0.130 g), which was used in the next step without any further purification. HRMS (TOF) (m/z): $[M+H]^+$ calcd for $\text{C}_{30}\text{H}_{44}\text{N}_5\text{O}_3\text{S}_2$, 586.2880; found, 586.2856. Compound **26c** was obtained according to GP2 as a dark red glass (0.086 g, 69%). ^1H NMR (CDCl_3 , 500 MHz) 0.00 (s, 9H), 0.04 (s, 9H), 0.96-1.00 (m, 4H), 1.02-1.04 (m, 8H), 1.21-1.24 (m, 4H), 1.53-1.55 (m, 4H), 2.10-2.13 (m, 3H), 2.21 (app br s, 4H), 2.34-2.36 (m, 5H), 2.57-2.58 (m, 2H), 2.68-2.69 (m, 2H), 2.72-2.75 (m, 2H), 3.40-3.52 (m, 4H), 3.92 (m, 3H), 4.17-4.20 (m, 2H), 4.26 (t, $J = 8.2$ Hz, 2H), 4.58 (s, 2H), 4.64-4.68 (m, 1H), 5.59 (br s, 1H), 5.97 (s, 1H), 6.21 (s, 1H), 6.56 (d, $J = 7.5$ Hz, 2H), 6.70 (s, 2H), 6.83 (s, 2H), 7.25-7.26 (m, 2H), 7.35 (d, $J = 6.0$ Hz, 1H), 7.62 (d, $J = 7.5$ Hz, 2H), 8.74 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) -1.4 (6C), 12.4, 14.9, 17.5, 17.7, 24.3, 25.8, 28.1, 29.1, 29.2, 29.4 ($\times 2$), 32.8, 36.6, 37.9, 38.0, 38.6, 42.8, 46.9, 52.9, 58.7, 64.1, 66.0, 95.7, 110.9, 111.8, 112.2 (2C), 114.0, 122.5, 123.4, 123.8, 126.4, 127.7, 129.2 (2C), 130.0, 139.9, 142.1, 149.3, 149.8, 150.4, 151.8, 154.1, 154.8, 159.8, 161.3, 167.6, 168.5, 168.6, 172.6, 173.2, 174.0, 198.1 (1 carbon atom unaccounted

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

for). HRMS (TOF) (m/z): $[(M+H+Na)/2]^{2+}$ calcd for $C_{60}H_{85}N_{12}Na_1O_{10}S_2Si_2$, 638.2689; found, 638.2663.

General procedure 3 (GP3): A solution of (1 eq.) Teoc-FA-LINKERS-prod. (1 eq.) in anhydrous DMSO (0.1 M) was treated with TBAF (1 M solution in THF, 2 eq.) and AcOH (1.25/0.1 mmol). The resulting reaction mixture was stirred at room temperature for 18 h under N_2 atmosphere. EtOAc was added to the reaction mixture until a precipitate formed, which was filtered using Milipore® and washed 3 times with water. The obtained dark red solid was dissolved in DMSO (0.1 M), treated with NaOH (0.2 M solution in MeOH) and stirred at room temperature for 10 min. Water was added and a precipitate was formed. The resulting sodium salt was isolated via microfiltration, then washed 3 times with water and dried under vacuum.

FA-DA-prod(C2) (27a)

Compound **27a** was obtained according to GP3 as dark red solid (0.040 g, 53%). M.p.: decomp. >230 °C. 1H NMR (DMSO- d_6 , 500 MHz, 80 °C) 1.39 (s, 3H), 1.96-2.22 (m, 7H), 2.68 (s, 3H), 3.00 (s, 4H), 3.90 (s, 3H), 4.32 (br s, 1H), 4.49 (s, 2H), 6.16 (br s, 1H), 6.26 (br s, 1H), 6.67 (br s, 4H), 6.78 (br s, 1H), 7.09 (br s, 1H), 7.64 (br s, 3H), 7.90 (br s, 1H), 8.65 (br s, 1H), 11.61 (br s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) 11.8, 15.4, 26.8, 28.2, 29.4, 32.0, 36.6, 37.3, 38.4 (2C), 45.9, 52.3, 58.4, 96.3, 110.2, 111.2 (2C), 113.1, 121.4, 122.1, 122.9, 127.6, 127.9, 128.2, 128.9 (2C), 140.9, 142.0, 148.5, 150.7, 153.9, 155.6, 156.3, 159.4, 161.2, 166.3, 167.4, 171.8, 174.0, 195.3 (1 carbon uncounted for). HRMS (TOF) (m/z): $[(M-H-Na)/2]^{2-}$ calcd for $C_{41}H_{42}NaN_{12}O_8$, 415.1630; found, 415.1648.

FA-DA-prod(C4) (27b)

Compound **27b** was obtained according to GP3 as dark red solid (0.036 g, 68%). M.p.: decomp. >200 °C. 1H NMR (DMSO- d_6 , 500 MHz, 60 °C) 1.54 (br s, 4H), 1.85-1.95 (m, 2H),

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

2.08-2.27 (m, 6H), 2.34 (s, 3H), 2.68 (s, 3H), 2.73 (br s, 2H), 3.06 (br s, 4H), 3.87 (s, 3H), 4.23-4.31 (m, 1H), 4.47 (d, 2H, $J = 5.6$ Hz), 6.19 (s, 1H), 6.24 (br s, 1H), 6.64 (d, 2H, $J = 8.6$ Hz), 6.69 (s, 1H), 6.78-6.79 (m, 1H), 6.87-6.91 (m, 2H), 6.78 (br s, 1H), 7.13 (br s, 1H), 7.65 (d, 2H, $J = 8.4$ Hz), 7.79-7.85 (m, 2H), 8.15 (d, 1H, $J = 5.4$ Hz), 8.63 (s, 1H), 11.81 (br s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) 11.8, 15.3, 23.4, 24.9, 26.6, 28.2, 32.0, 35.5, 38.3, 38.5, 41.6, 45.9, 52.1, 58.4, 96.3, 110.2, 111.2 (2C), 113.1, 121.4, 122.3, 122.9, 126.3, 127.6, 127.9, 128.2, 128.9 (2C), 140.8, 142.1, 148.5, 150.8, 153.8, 156.3, 159.3, 161.0, 161.1, 166.3, 167.4, 171.7, 172.2, 173.9, 196.3. HRMS (TOF) (m/z): $[\text{M}-\text{H}-\text{Na}/2]^{2-}$ calcd for $\text{C}_{43}\text{H}_{46}\text{NaN}_{12}\text{O}_8$, 429.1786; found, 429.1796.

FA-DA-prod(C8) (27c)

Compound **27c** was obtained according to GP3 as a black solid (0.025 g, 67%). M.p.: decomp. > 205 °C. ^1H NMR (DMSO- d_6 , 500 MHz) 1.24-1.27 (m, 8H), 1.45-1.48 (m, 2H), 1.55-1.57 (m, 2H), 1.88-1.93 (m, 1H), 2.01-2.09 (m, 3H), 2.15-2.19 (m, 2H), 2.34 (s, 3H), 2.67 (s, 3H), 2.72 (t, 2H, $J = 7.0$ Hz), 3.00-3.10 (m, 5H), 3.30 (br s, 4H), 3.87 (s, 3H), 4.28-4.30 (m, 1H), 4.48 (d, 2H, $J = 5.5$ Hz), 6.19 (s, 1H), 6.24 (s, 1H), 6.64 (d, 2H, $J = 8.2$ Hz), 6.69 (s, 1H), 6.79 (s, 1H), 6.89-6.91 (m, 2H), 7.14 (s, 1H), 7.65 (d, 2H, $J = 8.2$ Hz), 7.74 (s, 1H), 7.84 (s, 1H), 8.17 (d, 1H, $J = 7.5$ Hz), 8.64 (s, 1H), 11.43 (s, 1H), 11.81 (s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) 11.7, 15.3, 23.7, 25.1, 27.0, 28.2, 28.7 ($\times 2$), 28.9, 32.0, 35.4, 41.8, 45.9, 52.3, 58.4, 77.5, 96.2, 110.1, 111.2 (2C), 113.0, 121.5, 122.4, 122.9, 126.3, 127.5, 127.9, 128.2, 128.8 (2C), 140.7, 142.0, 148.5, 150.7, 154.0, 155.6, 156.2, 159.3, 161.2, 166.1, 167.3, 171.8, 172.3, 173.0, 174.2, 196.6 (1 carbon uncounted for). HRMS (TOF) (m/z): $[(\text{M}+\text{H}-\text{Na})]^+$ calcd for $\text{C}_{47}\text{H}_{55}\text{NaN}_{12}\text{O}_8$, 915.4271; found, 915.4232.

FA-PEG-prod(C4) (28a)

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

Compound **28a** was obtained according to GP3 as a black solid (0.010 g, 41%). M.p.: 185 °C. ^1H NMR (DMSO- d_6 , 500 MHz) 1.57-1.63 (m, 4H), 1.86-1.93 (m, 1H), 2.01-2.07 (m, 1H), 2.17 (t, 2H, $J = 7.5$ Hz), 2.35 (s, 3H), 2.40 (t, 2H, $J = 6.7$ Hz), 2.69 (s, 3H), 2.97 (t, 2H, $J = 6.7$ Hz), 3.04-3.10 (m, 4H), 3.34-3.50 (m, 12H), 3.87 (s, 3H), 4.24-4.28 (m, 1H), 4.48 (d, 2H, $J = 5.5$ Hz), 6.19 (s, 1H), 6.24 (s, 1H), 6.64 (d, 2H, $J = 8.7$ Hz), 6.70 (s, 1H), 6.79 (s, 1H), 6.88-6.90 (m, 2H), 7.13 (s, 1H), 7.64 (d, 2H, $J = 8.7$ Hz), 7.78-7.81 (m, 2H), 8.12 (d, 1H, $J = 7.0$ Hz), 8.64 (s, 1H), 11.82 (br s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) 11.8, 15.4, 26.6, 29.3, 29.4 (2C), 32.0, 35.8, 37.3 (2C), 45.9, 52.3, 58.4, 68.0 (2C), 68.1, 69.5, 69.7 (4C), 96.3, 110.1, 111.2 (2C), 113.1, 121.4, 122.1, 122.9, 126.3, 127.6, 127.9, 128.2, 128.9 (2C), 140.8, 142.1, 148.5, 150.7, 153.8, 159.4, 166.2, 167.4, 171.5, 173.9, 195.3 (3 carbon atoms unaccounted for). HRMS (TOF) (m/z): $[(\text{M}-\text{H}-\text{Na})/2]^{2-}$ calcd for $\text{C}_{49}\text{H}_{58}\text{NaN}_{12}\text{O}_{11}$, 495.2179; found, 495.2177.

FA-PEG-prod(C4) (28b)

Compound **28b** was obtained according to GP3 as dark red solid (0.065 g, 79%). M.p.: decomp. >170 °C. ^1H NMR (DMSO- d_6 , 500 MHz) 1.55-1.64 (m, 8H), 1.89-1.93 (m, 1H), 2.02-2.10 (m, 3H), 2.15-2.19 (m, 2H), 2.35 (s, 3H), 2.68 (s, 3H), 2.73 (br s, 2H), 3.02-3.10 (m, 4H), 3.36 (dd, 4H, $J = 12.6, 6.3$ Hz), 3.45-3.48 (m, 8H), 3.87 (s, 3H), 4.23-4.30 (m, 1H), 4.48 (d, 2H, $J = 5.7$ Hz), 6.19 (s, 1H), 6.24 (br s, 1H), 6.64 (d, 2H, $J = 8.6$ Hz), 6.70 (s, 1H), 6.80 (br s, 1H), 6.88-6.91 (m, 2H), 7.13 (s, 1H), 7.65 (d, 2H, $J = 8.6$ Hz), 7.73-7.80 (m, 2H), 8.14 (d, 1H, $J = 7.4$ Hz), 8.64 (s, 1H), 11.81 (br s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) 11.8, 15.33, 23.5, 25.1, 26.7, 29.3, 29.4 (2C), 32.0, 35.5, 35.7, 35.8 (2C), 41.2, 41.6, 45.9, 58.4, 68.0 (2C), 68.1, 69.5, 69.7 (4C), 96.3, 110.2, 111.2 (2C), 113.1, 121.4, 122.3, 122.9, 126.3, 128.2, 128.9 (2C), 140.8, 142.1, 148.5, 148.6, 150.7, 153.8, 159.4, 161.0, 166.2, 167.4, 171.5, 172.0, 173.9, 196.5. HRMS (TOF) (m/z): $[\text{M}-\text{Na}]^-$ calcd for $\text{C}_{51}\text{H}_{63}\text{NaN}_{12}\text{O}_{11}$, 1019.4745; found, 1019.4718.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

FA-PEG-prod(C8) (28c)

Compound **28c** was obtained according to GP3 as dark red solid (0.021 g, 38%). M.p.: decomp. >150 °C. ¹H NMR (DMSO-d₆, 500 MHz) 1.25-1.26 (br m, 8H), 1.44-1.50 (m, 2H), 1.53-1.64 (m, 4H), 2.03 (t, 2H, *J* = 7.1 Hz), 2.14-2.28 (m, 4H), 2.34 (s, 3H), 2.67 (s, 3H), 2.72 (t, 2H, *J* = 7.1 Hz), 3.06 (dd, 4H, *J* = 12.9, 6.8 Hz), 3.33-3.39 (m, 4H), 3.43-3.49 (m, 8H), 3.87 (s, 3H), 4.22-4.29 (m, 1H), 4.48 (d, 2H, *J* = 5.7 Hz), 6.19 (s, 1H), 6.64 (d, 2H, *J* = 8.3 Hz), 6.69 (s, 1H), 6.78-6.80 (m, 1H), 6.85-6.92 (m, 3H), 7.13 (br s, 1H), 7.64 (d, 2H, *J* = 8.3 Hz), 7.69-7.72 (m, 1H), 7.77-7.81 (m, 1H), 8.14 (d, 1H, *J* = 7.0 Hz), 8.64 (s, 1H), 11.43 (br s, 1H), 11.81 (br s, 1H). ¹³C NMR (DMSO-d₆, 125 MHz) 11.8, 15.3, 23.8, 25.3, 26.7, 28.6, 28.7, 28.8, 29.0, 29.3, 29.4, 32.0, 35.4, 35.7, 35.8, 41.8, 45.9, 52.3, 58.4, 68.0, 68.1, 69.5 (×2), 69.7 (4C), 96.3, 110.2, 111.2 (2C), 113.1, 121.4, 122.4, 122.9, 126.3, 127.5, 127.9, 128.2, 128.9 (2C), 140.7, 142.1, 148.5, 148.6, 150.7, 153.8, 153.9, 159.4, 166.2, 167.4, 171.5, 172.0, 173.8, 197.7. HRMS (TOF) (*m/z*): [(M-H-Na)/2]⁻ calcd for C₅₅H₇₀NaN₁₂O₁₁, 537.2649; found, 537.2664.

FA-SS-prod(C4) (29a)

Compound **29a** was obtained according to GP3 as a dark red solid (0.009 g, 26%). ¹H NMR (DMSO-d₆, 300 MHz, 50 °C) 1.86-1.96 (m, 1H), 2.03-2.17 (m, 1H), 2.19-2.23 (m, 2H), 2.35 (s, 3H), 2.42 (t, 2H, *J* = 6.6 Hz), 2.68 (s, 3H), 2.72-2.96 (m, 4H), 3.00 (t, 1H, *J* = 7.0 Hz), 3.30-3.36 (m, 5H), 3.89 (s, 3H), 4.29 (br s, 1H), 4.49 (d, 1H, *J* = 4.5 Hz), 6.20 (s, 1H), 6.25 (s, 1H), 6.62-6.70 (m, 3H), 6.89-6.93 (m, 3H), 7.04-7.07 (m, 1H), 7.13 (s, 1H), 7.65 (d, 2H, *J* = 7.5 Hz), 8.06 (t, 1H, *J* = 5.7 Hz), 8.15-8.17 (m, 1H), 8.64 (s, 1H), 11.82 (br s, 1H). ¹³C NMR (DMSO-d₆, 125 MHz) 11.8, 15.4, 26.5, 28.2, 29.3, 31.9, 37.2 (2C), 37.3, 37.9, 38.1 (2C), 39.0, 45.9, 52.1, 58.4, 96.3, 110.1 (2C), 111.1, 113.1, 121.3, 122.1, 122.9, 126.3, 127.6, 127.9, 128.1, 128.9 (2C), 140.9, 142.0, 148.6, 150.7, 153.8, 159.3, 166.2, 167.3, 171.7 (×2), 173.8, 195.1 (1 carbon atom)

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

unaccounted for). HRMS (TOF) (m/z): $[(M-H-Na)/2]^{2-}$ calcd for $C_{43}H_{46}NaN_{12}O_8S_2$, 461.1507; found, 461.1530.

FA-SS-prod(C4) (29b)

Compound **29b** was obtained according to GP3 as dark red solid (0.047 g, 30%). M.p.: decomp. >210 °C. 1H NMR (DMSO- d_6 , 500 MHz) 1.55 (br s, 4H), 1.84-1.96 (m, 2H), 2.02-2.12 (m, 2H), 2.16-2.22 (m, 2H), 2.35 (s, 3H), 2.68 (s, 3H), 2.73-2.78 (m, 6H), 3.29-3.32 (m, 4H), 3.87 (s, 3H), 4.23-4.32 (m, 1H), 4.48 (d, 2H, $J = 5.5$ Hz), 6.19 (s, 1H), 6.24 (br s, 1H), 6.66 (d, 2H, $J = 8.6$ Hz), 6.80 (br s, 1H), 6.88-6.93 (m, 2H), 6.99 (br s, 1H), 7.13 (br s, 1H), 7.64 (dd, 2H, $J = 8.6, 2.8$ Hz), 7.96-8.04 (m, 2H), 8.09-8.16 (m, 1H), 8.64 (s, 1H), 11.81 (br s, 1H). ^{13}C NMR (DMSO- d_6 , 125 MHz) 11.8, 15.3, 23.4, 25.0, 26.6, 32.0, 35.4, 37.2 ($\times 2$), 37.3, 37.9, 38.0, 41.6, 45.9, 52.3, 58.4, 96.3, 110.2, 111.2 (2C), 113.1, 121.4, 122.4, 122.9, 126.3, 127.9, 128.2, 128.9 (2C), 140.8, 142.1, 148.5, 150.7, 153.8, 156.3, 159.3, 161.1, 166.0, 166.2, 166.3, 167.3, 171.8, 172.3, 173.9, 196.5. HRMS (TOF) (m/z): $[M-Na]^-$ calcd for $C_{45}H_{51}NaN_{12}O_8S_2$, 951.3400; found, 951.3369.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

Acknowledgments

During this work, E.M. was supported by a trainee award from The Beatrice Hunter Cancer Research Institute (BHCRI) with funds provided by Cancer Care Nova Scotia as part of The Terry Fox Foundation Strategic Health Research Training Program in Cancer Research at Canadian Institutes of Health Research (CIHR). This work was funded by research grants to A.T. from: CIHR (Grant # 133110), Canadian Breast Cancer Foundation-Atlantic, Breast Cancer Society of Canada/QEII Foundation Awards for Breast Cancer Research, BHCRI and Nova Scotia Health Research Foundation. A.T. is a Senior Scientist of the BHCRI.

References

1. A. Fürstner, *Angew. Chem. Int. Ed.*, 2003, 42, 3582-3603.
2. N. R. Williamson, P. C. Fineran, F. J. Leeper and G. P. Salmond, *Nat. Rev. Microbiol.*, 2006, 4, 887-899.
3. R. K. Suryawanshi, C. D. Patil, H. P. Borase, C. P. Narkhede, A. Stevenson, J. E. Hallsworth and S. V. Patil, *Int. J. Cosmet. Sci.*, 2015, 37, 98-107.
4. Y. Kim and J. Choi, *Fiber Polym.*, 2015, 16, 1981-1987.
5. Y. Ren, J. Gong, R. Fu, Z. Li, Q. Li, J. Zhang, Z. Yu and X. Cheng, *Dyes Pigm.*, 2017, 138, 147-153.
6. A. Fürstner and E. J. Grabowski, *ChemBioChem*, 2001, 2, 706-709.
7. A. Fürstner, K. Reinecke, H. Prinz and H. Waldmann, *ChemBioChem*, 2004, 5, 1575-1579.
8. M. S. Melvin, J. T. Tomlinson, G. Park, C. S. Day, G. R. Saluta, G. L. Kucera and R. A. Manderville, *Chem. Res. Toxicol.*, 2002, 15, 734-741.
9. M. S. Melvin, J. T. Tomlinson, G. R. Saluta, G. L. Kucera, N. Lindquist and R. A. Manderville, *J. Am. Chem. Soc.*, 2000, 122, 6333-6334.
10. B. Montaner, W. Castillo-Avila, M. Martinell, R. Oellinger, J. Aymami, E. Giralt and R. Perez-Tomas, *Toxicol. Sci.*, 2005, 85, 870-879.
11. S. Ohkuma, T. Sato, M. Okamoto, H. Matsuya, K. Arai, T. Kataoka, K. Nagai and H. H. Wasserman, *Biochem. J.*, 1998, 334, 731-741.
12. G. Park, J. T. Tomlinson, M. S. Melvin, M. W. Wright, C. S. Day and R. A. Manderville, *Org. Lett.*, 2003, 5, 113-116.
13. R. I. Sáez Díaz, S. M. Bennett and A. Thompson, *ChemMedChem*, 2009, 4, 742-745.
14. T. Sato, H. Konno, Y. Tanaka, T. Kataoka, K. Nagai, H. H. Wasserman and S. Ohkuma, *J. Biol. Chem.*, 1998, 273, 21455-21462.
15. J. L. Seganish and J. T. Davis, *Chem. Commun.*, 2005, 5781-5783.
16. W. R. Hearn, M. K. Elson, R. H. Williams and J. Medina-Castro, *J. Org. Chem.*, 1970, 35, 142-146.
17. E. Marchal, S. Rastogi, A. Thompson and J. T. Davis, *Org. Biomol. Chem.*, 2014, 12, 7515-7522

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

18. E. Marchal, D. A. Smithen, I. M. Uddin, A. W. Robertson, D. L. Jakeman, V. Mollard, C. D. Goodman, K. S. MacDougall, S. A. McFarland, G. I. McFadden and A. Thompson, *Org. Biomol. Chem.*, 2014, 12, 4132-4142.
19. E. Marchal, M. I. Uddin, D. A. Smithen, L. A. Hawco, M. Lanteigne, D. P. Overy, R. G. Kerr and A. Thompson, *RSC Adv.*, 2013, 3, 22967-22971
20. S. Rastogi, E. Marchal, I. Uddin, B. Groves, J. Colpitts, S. A. McFarland, J. T. Davis and A. Thompson, *Org. Biomol. Chem.*, 2013, 11, 3834-3845.
21. J. Regourd, A. Al-Sheikh Ali and A. Thompson, *J. Med. Chem.*, 2007, 50, 1528-1536.
22. R. I. Sáez Díaz, J. Regourd, P. V. Santacroce, J. T. Davis, D. L. Jakeman and A. Thompson, *Chem. Commun.*, 2007, 2701-2703.
23. D. A. Smithen, A. M. Forrester, D. P. Corkery, G. Dellaire, J. Colpitts, S. A. McFarland, J. N. Berman and A. Thompson, *Org. Biomol. Chem.*, 2013, 11, 62-68.
24. M. I. Uddin, S. Thirumalairajan, S. M. Crawford, T. S. Cameron and A. Thompson, *Synlett*, 2010, 2561-2564.
25. C. Ferrario and G. Batist, *Expert. Opin. Drug Dis.*, 2014, 9, 647-668.
26. A. Cheung, H. J. Bax, D. H. Josephs, K. M. Ilieva, G. Pellizzari, J. Opzoomer, J. Bloomfield, M. Fittall, A. Grigoriadis, M. Figini, S. Canevari, J. F. Spicer, A. N. Tutt and S. N. Karagiannis, *Oncotarget*, 2016, 7, 52553-52574.
27. R. Paulmurugan, R. Bhethanabotla, K. Mishra, R. Devulapally, K. Foygel, T. Sekar, J. Ananta, T. Massoud and A. Joy, *Mol. Cancer Ther.*, 2016, 15, 221-231.
28. Y. Lu and P. S. Low, *Adv. Drug Deliv. Rev.*, 2002, 54, 675-693.
29. J. Sudimack and R. J. Lee, *Adv. Drug Deliv. Rev.*, 2000, 41, 147-162.
30. F. Zagouri, M. A. Dimopoulos, E. Bournakis and C. A. Papadimitriou, *Eur. J. Gynaecol. Oncol.*, 2010, 31, 268-277.
31. Y.-S. Yi, *Immune Netw.*, 2016, 16, 337-343.
32. W. Xia, A. R. Hilgenbrink, E. L. Matteson, M. B. Lockwood, J. X. Cheng and P. S. Low, *Blood*, 2009, 113, 438-446.
33. L. H. Matherly, Z. Hou and Y. Deng, *Cancer Metastasis Rev.*, 2007, 26, 111-128.
34. J. M. Scott and D. G. Weir, *J. Cardiovasc. Risk*, 1998, 5, 223-227.
35. R. Zhao, S. H. Min, Y. Wang, E. Campanella, P. S. Low and I. D. Goldman, *J. Biol. Chem.*, 2009, 284, 4267-4274.
36. P. S. Low and S. A. SuKularatne, *Curr. Opin. Chem. Biol.*, 2009, 13, 256-262.
37. S. Wang and P. S. Low, *J. Control. Release.*, 1998, 53, 39-48.
38. C. L. A. Hawco, E. Marchal, M. I. Uddin, A. E. G. Baker, D. P. Corkery, G. Dellaire and A. Thompson, *Bioorg. Med. Chem.*, 2013, 21, 5995-6002.
39. G. Huang, D. Pemp, P. Stadtmüller, M. Nimczick, J. Heilmann and M. Decker, *Bioorg. Med. Chem. Lett.*, 2014, 24, 4209-4214.
40. S. Dhar, Z. Liu, J. Thomale, H. Dai and S. J. Lippard, *J. Am. Chem. Soc.*, 2008, 130, 11467-11476.
41. S. Zalipsky, *Adv. Drug Deliv. Rev.*, 1995, 16, 157-182.
42. M. P. Grillo, *Expert Opin. Drug Metab. Toxicol.*, 2015, 11, 1281-1302.
43. H. Joyce, A. McCann, M. Clynes and A. Larkin, *Expert Opin. Drug Metab. Toxicol.*, 2015, 11, 795-809.
44. A. Sullivan, A. Gibson, B. K. Park and D. J. Naisbitt, *Expert Opin. Drug Metab. Toxicol.*, 2015, 11, 357-368.
45. R. S. Shirazi, K. K. Ewert, C. Leal, R. N. Majzoub, N. F. Boussein and C. R. Safinya, *Biochim. Biophys. Acta – Biomembranes*, 2011, 1808, 2156-2166.
46. I. R. Vlahov and C. P. Leamon, *Bioconjugate Chem.*, 2012, 23, 1357-1369.

Step-wise synthetic approach is necessary to access γ -conjugates of folate:

folate-conjugated prodigiosenes

47. C. Chen, J. Ke, X. E. Zhou, W. Yi, J. S. Brunzelle, J. Li, E.-L. Yong, H. E. Xu and K. Melcher, *Nature*, 2013, 500, 486-489.
48. M. P. Carrasco, E. A. Enyedy, N. I. Krupenko, S. A. Krupenko, E. Nuti, T. Tuccinardi, S. Santamaria, A. Rossello, A. Martinelli and M. A. Santos, *Med. Chem.*, 2011, 7, 265-274.
49. S. K. Choi, T. Thomas, M.-H. Li, A. Kotlyar, D. A. and J. R. J. Baker, *Chem. Commun.*, 2010, 46, 2632-2634.
50. A. Galbiati, C. Tabolacci, B. M. D. Rocca, P. Mattioli, S. Beninati, G. Paradossi and A. Desideri, *Bioconjugate Chem.*, 2011, 22.
51. L. Jiang, Z.-m. Gao, L. Ye, A.-y. Zhang and Z.-g. Feng, *Polymer*, 2013, 54, 5188-5198.
52. H. Jing, Z. Guo, W. Guo, W. Yang, P. Xu and X. Zhang, *Bioorg. Med. Chem. Lett.*, 2012, 22, 3418-3424.
53. C. Lainé, E. Mornet, L. Lemiègre, T. Montier, S. Cammas-Marion, C. Neveu, N. Carmoy, P. Lehn and T. Benvegnu, *Chemistry*, 2008, 14, 8330-8340.
54. P. Li, Y. Wang, F. Zeng, L. Chen, Z. Peng and L. X. Kong, *Carbohydr. Res.*, 2011, 346, 801-806.
55. M. A. Santos, E. A. Enyedy, E. Nuti, A. Rossello, N. I. Krupenko and S. A. Krupenko, *Bioorg. Med. Chem.*, 2007, 15, 1266-1274.
56. R. K. Singh, D. Rai, D. Yadav, A. Bhargava, J. Balzarini and E. De Clercq, *Eur. J. Med. Chem.*, 2010, 45, 1078-1086.
57. A. F. Trindade, R. F. M. Frade, E. M. S. Macoas, C. Graca, C. A. B. Rodrigues, J. M. G. Martinho and C. A. M. Afonso, *Org. Biomol. Chem.*, 2014, 12, 3181-3190.
58. P. M. Valencia, M. H. Hanewich-Hollatz, W. Gao, F. Karim, R. Langer, R. Karnik and O. C. Farokhzad, *Biomaterials*, 2011, 32, 6226-6233.
59. Y. Yang, Y. M. Zhang, Y. Chen, D. Zhao, J. T. Chen and Y. Liu, *Chemistry*, 2012, 18, 4208-4215.
60. H. Zhang, Z. Cai, Y. Sun, F. Yu, Y. Chen and B. Sun, *J. Biomed. Mater. Res. Part A*, 2012, 100A, 2441-2449.
61. A. Bettio, M. Honer, C. Müller, M. Brühlmeier, U. Müller, R. Schibli, V. Groehn, A. P. Schubiger and S. M. Ametamey, *J. Nucl. Med.*, 2006, 47, 1153-1160.
62. I. R. Vlahov, H. K. Santhapuram, P. J. Kleindl, S. J. Howard, K. Stanford and C. P. Leamon, *Bioorg. Med. Chem. Lett.*, 2006, 16, 5093-5096.
63. W. A. Henne, S. A. Kularatne, J. Hakenjos, J. D. Carron and K. L. Henne, *Bioorg. Med. Chem. Lett.*, 2013, 23, 5810-5813.
64. T. Suzuki, S. Hisakawa, Y. Itoh, N. Suzuki, K. Takahashi, M. Kawahata, K. Yamaguchi, H. Nakagawa and N. Miyata, *Bioorg. Med. Chem. Lett.*, 2007, 17, 4208-4212.
65. M. Nomura, S. Shuto and A. Matsuda, *J. Org. Chem.*, 2000, 65, 5016-5021.
66. C. P. Leamon, M. A. Parker, I. R. Vlahov, L. C. Xu, J. A. Reddy, M. Vetzal and N. Douglas, *Bioconjug. Chem.*, 2002, 13, 1200-1210.
67. J. D. Seitz, J. G. Vineberg, E. Herlihy, B. Park, E. Melief and I. Ojima, *Bioorg. Med. Chem.*, 2015, 23, 2187-2194.
68. E. Vlashi, L. E. Kelderhouse, J. E. Sturgis and P. S. Low, *ACS Nano.*, 2013, 7, 8573-8582.
69. J. Luo, S. M.D., D. A. Lantrip, S. Wang and P. L. Fuchs, *J. Am. Chem. Soc.*, 1997, 119, 10004-10013.