Communication

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Process Development of the Synthesis and Purification of a Reactive Immuno-PET Conjugate Intermediate

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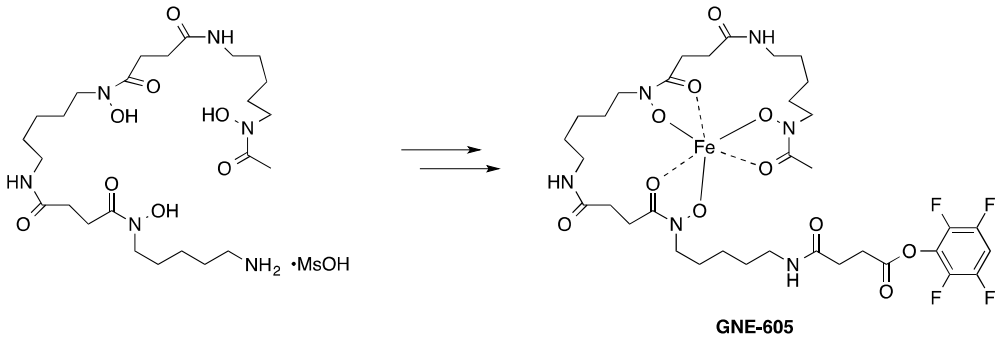
TOC GRAPHIC



ABSTRACT

We describe a practical multi-gram scale synthesis of the reactive Immuno-PET linker-chelator **GNE-605** from the commercially available starting material desferrioxamine B mesylate. A modified 3-step synthetic procedure was developed that significantly reduces the amount of solvents and reagents required as compared to previous literature. Additionally, due to the unique handling challenges posed by this reactive intermediate, a liquid chromatography purification and lyophilization procedure was developed to allow for the first isolation of **GNE-605** as a solid material.

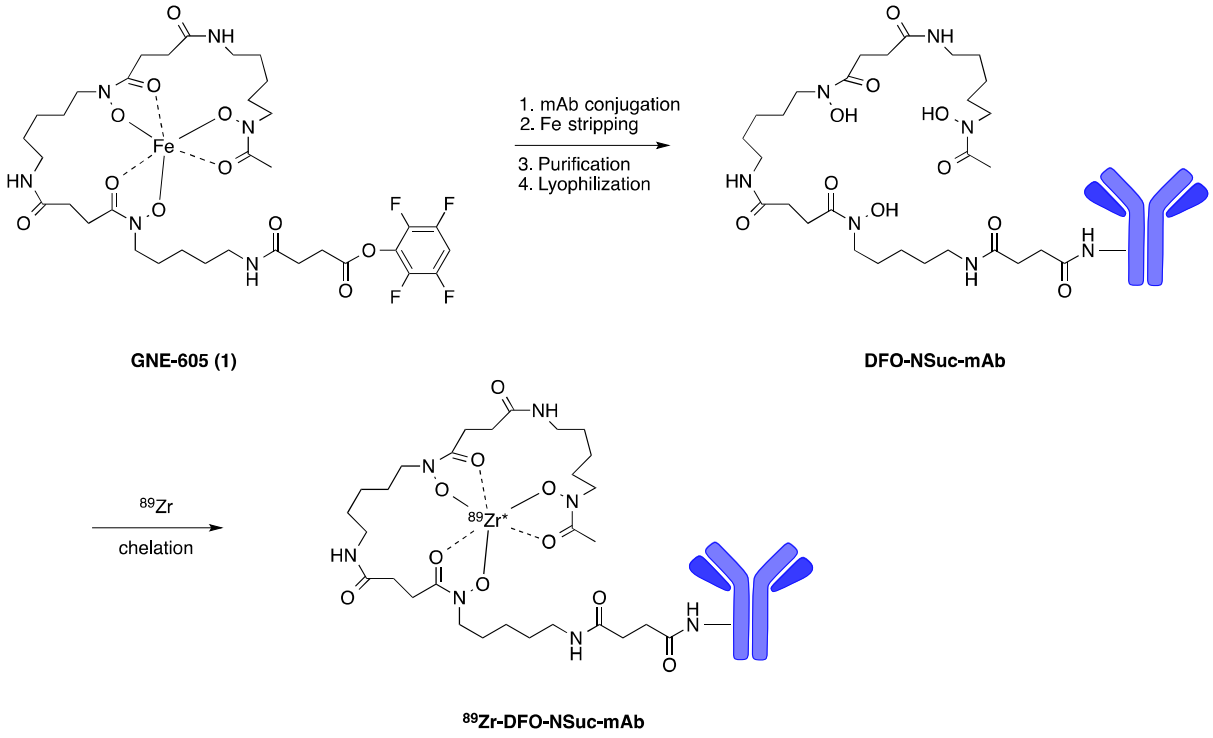
KEYWORDS: Immuno-PET, radioimmunoconjugate, ADC, conjugate

INTRODUCTION

Positron emission tomography (PET) is a valuable tool for studying the uptake of molecules within the body with wide ranging applications in oncology, cardiology, neuroimaging and pharmacokinetics. By using a positron-emitting radionuclide such as ^{18}F, ^{89}Zr or ^{67}Ga as a tracer, a three-dimensional image can be generated to show where the tracer is located within the body. A new class of non-therapeutic antibody small molecule conjugates known as Immuno-PET are of increasing interest to the drug development community due to their potential application as an imaging tool in the clinic.[1] While a number of platforms have been developed, the ^{89}Zr chelating PET ligand DFO-NSuc-mAb (Scheme 1) was of interest for development as a generic platform that could enable real time imaging of monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs) in the clinic. With typical dosing of the conjugate ranging from 5-50 mg, a single gram of the small molecule is sufficient to provide multi-kilogram quantities of conjugate depending on the expected drug-antibody ratio (DAR). A generic scheme of the Immuno-PET synthesis is given in Scheme 1. The first step in the Immuno-PET synthesis involves preparation of the small molecule linker chelator **GNE-605**, also referred to as DFO-Suc-TFP-Fe, then conjugation to the antibody of choice, iron stripping, purification and lyophilization afford the non-chelated Immuno-PET conjugate (DFO-NSuc-mAb). Final chelation with ^{89}Zr gives the active Immuno-PET conjugate ^{89}Zr-DFO-NSuc-TFP. In order to successfully implement the Immuno-PET platform, we first needed to access the reactive linker chelator **GNE-605** and prepare gram quantities of this compound, which had previously only been synthesized on milligram scale.

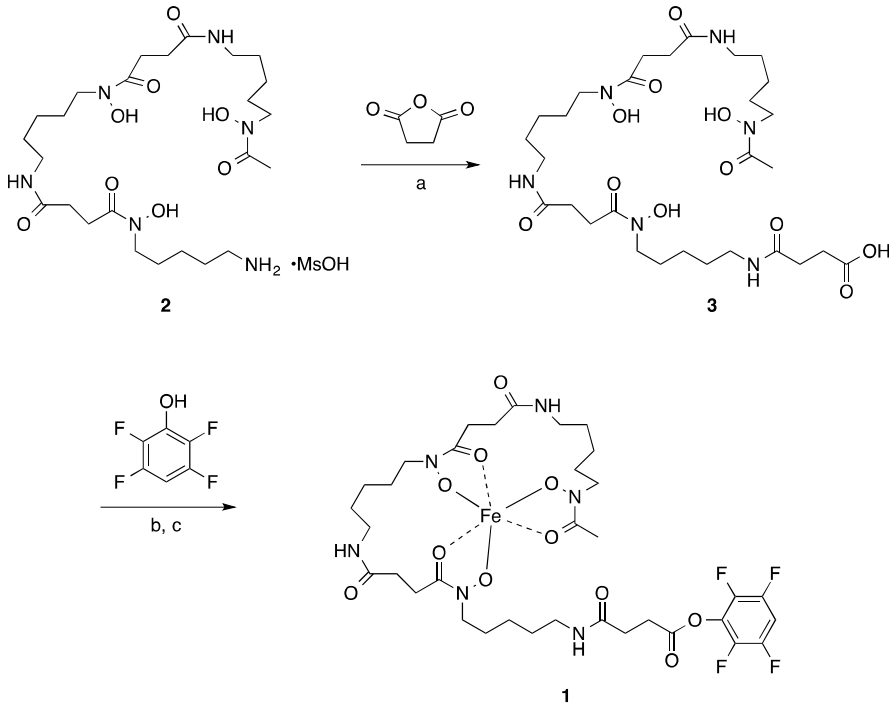
RESULTS AND DISCUSSION

Scheme 1. Immuno-PET Imaging Agent Synthesis



The published synthesis[2] of the linker-chelator **GNE-605 (1)** is given in Scheme 2. This three-step route starts with the commercially available mesylate salt of desferrioxamine B **2**,[3] a strong chelator of Fe(III) which has been approved for commercial use in the treatment of acute iron poisoning[4]. The terminal primary amine undergoes amidation in the presence of succinic anhydride and base to give the free acid **3**. Chelation with Fe(III) in the form of iron trichloride serves to protect the *N*-hydroxyamide functional groups and is followed by a 1-ethyl-3-(3dimethylaminopropyl)carbodiimide (EDC) mediated coupling with 2,3,5,6-tetrafluorophenol (TFP) to furnish the final compound **1**. The crude solution from this final reaction is then purified by preparative reverse-phase chromatography and the compound is taken on to the mAb conjugation step as a solution in acetonitrile.

Scheme 2. Literature Route to GNE-605 (1)[a]



[a] Reagents and conditions: (a) succinic anhydride, pyridine, 25 ˚C, 24 h; (b) $FeCl_3$ in 0.5 M HCl, 0.1 M Na_2CO_3(aq), 0.9% NaCl (aq), 25 ˚C; (c) 2,3,5,6-tetrafluorophenol, EDC•HCl, MeCN, 25 ˚C.

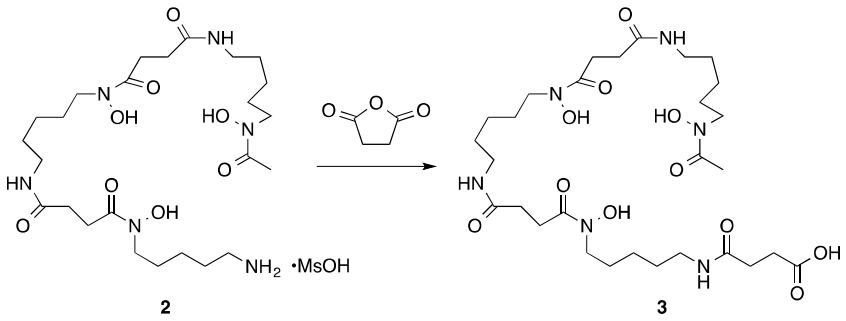
In replicating this literature route in our laboratories, we identified several key issues that would pose a challenge for multi-gram scale synthesis. The first step, installation of the succinate linker, used the undesirable solvent pyridine, a large excess of succinic anhydride (1000 mol%) and workup was accomplished by acidifying to cause product precipitation and washing with 1000 volumes of 0.01 M aqueous HCl solution, which would have equated to 20 L on 20 g scale. Additionally, due to the small particle size of the solid product **3**, the initial filtration and subsequent cake washes with water and heptane were exceedingly slow and resulted in a very dense cake. In the second step, the low solubility of the isolated acid **3**[5] again gave rise to the need to use very high solvent volumes (60 mL/g of aqueous 0.9% NaCl and 22 mL/g of aqueous 0.1 M Na_2CO_3) in order to completely dissolve the starting material and achieve complete chelation of Fe(III). For the final coupling step, large excesses of both the coupling reagent EDC•HCl (2900 mol%) and TFP (1000 mol%) were required to achieve complete conversion to the phenol, presumably as a result of hydrolysis of the coupling reagent as the reaction was performed in an aqueous media. The final and most critical issue facing the literature synthesis arose from the fact that compound **1** had only been produced on milligram scale[6] and had never been

isolated. As part of a mAb imaging project, we needed to develop synthesis and isolation conditions that would provide a minimum of 20 g of **GNE-605**. Our main focus on the process chemistry front was to develop new reaction conditions amenable to scale-up that would eliminate the use of aqueous reaction media, reduce solvent volumes and improve reagent stoichiometry. Clearly, both new purification and isolation methods that could produce gram quantities of **1** in a solid form were much needed.

RESULTS AND DISCUSSION

Process development for the succinate addition step to convert the DFO starting material **2** to the free acid **3** focused on removing the environmentally unfriendly solvent pyridine, reducing or eliminating the extensive aqueous work-up and avoiding the slow filtration during isolation. We evaluated a series of organic solvents (Table 1) and found that polar aprotic solvents performed well in this transformation. Both DMF and NMP performed better than pyridine, achieving 100% conversion to **3** after 16 hours (entries 7–8) as opposed to the 24 hours required in the literature system. These solvents also exhibited a greater solubilizing effect on the starting material which allowed the amount of solvent used to be reduced from 15 volumes to 10 volumes. Furthermore, increasing the temperature to 50 ˚C led to a faster reaction rate with complete conversion achieved in only two hours (entry 9). This increase in reaction rate allowed for additional process improvements such as reducing the amount of succinic anhydride used from 1000 mol% to only 115 mol%. The final modification to the reaction conditions was the addition of 120 mol% of *N,N*-disopropylethylamine (DIPEA) to effectively generate the freebase of the starting material, which had previously been accomplished by using pyridine as the reaction solvent. The use of large amounts of aqueous media to work-up the reaction and precipitate the product was avoided by telescoping the reaction mixture directly on to the chelation step. This also served to eliminate the extremely slow filtration that was observed when isolating **3**.

Table 1: Solvent Evaluation for Succinate Amidation[a]

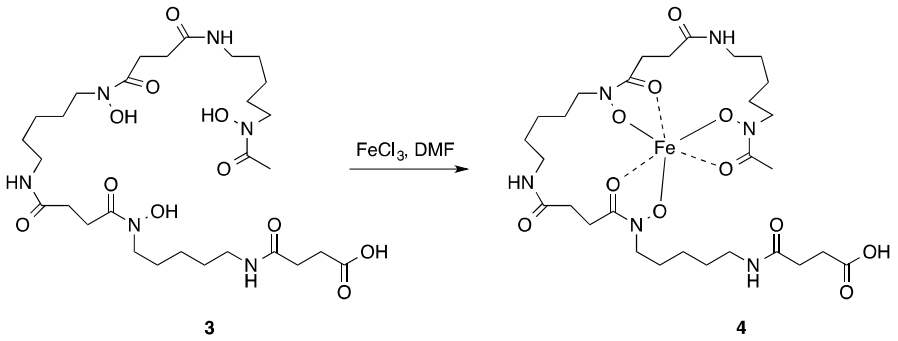


Entry	Solvent	Temp (˚C)	Time (h)	conversion(%)[b]
1	THF	25	48	70
2	IPA	25	48	66
3	EtOAc	25	48	49
4	iPrOAc	25	48	61
5	DCM	25	48	74
6	MeCN	25	48	82
7	NMP	25	16	100
8	DMF	25	16	100
9	DMF	50	2	100

[a] Reaction performed with **2** (25 mg, 38.1 μmol,100 mol%), succinic anhydride (77 mg, 77.0 μmol, 200 mol%), *N*,*N*-diisopropylethylamine(10 μL, 57.1 μmol 150 mol%) and solvent (0.5 mL, 20 mL/g) [b] Determined by HPLC analysis with UV detection at 220 nm.

Modification of the chelation conditions focused primarily on replacing the aqueous media used in the literature procedure with an organic medium that would be compatible with the telescoped material from the modified succinate amidation procedure. It was found that the addition of 100 mol% of $FeCl_3$ dissolved in 2.5 volumes of DMF at room temperature resulted in complete chelation to form chelated **4** after only 15 minutes (Scheme 3). The chelation could be monitored visually by a color change of the white slurry to dark red and also by HPLC assay showing 100% conversion to a new peak at 420 nm.[7] A corresponding increase in solubility was also noted at this stage. The resulting reaction mixture was used directly in the next stage.

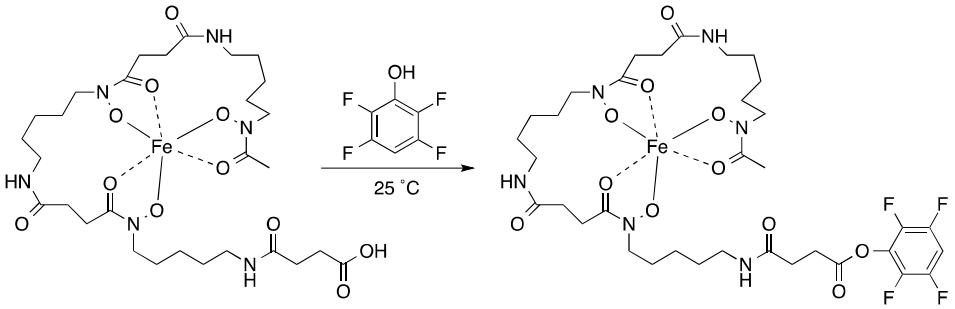
Scheme 3. Chelation with $FeCl_3$



3 **4**

For the installation of active TFP ester to generate product **1**, process development was focused on optimization of the coupling reagent, replacing the aqueous reaction media with an organic solvent and developing work-up conditions that would remove process impurities. Examining the reagent used in the literature procedure, EDC•HCl, it was found that the coupling reaction performed much better in DMF than NMP (Table 2, entries 1–2). Additional coupling reagents such as CDI and propanephosphonic acid anhydride (T3P) were also examined but neither resulted in high levels of product formation (Table 2, entries 3–6). In the case of T3P, the chelated starting material **4** underwent decomposition. In addition to changing the reaction media to DMF, the amount of EDC•HCl used was reduced from 2900 mol% to 200 mol% and the amount of TFP used was reduced from 1000 mol% to 200 mol%.[8] Neither change had a negative impact on the reaction rate as the reaction reached completion in 5 hours at 25 ˚C (Table 2, entry 7).[9]

Table 2. Evaluation of Coupling Reagents[a]



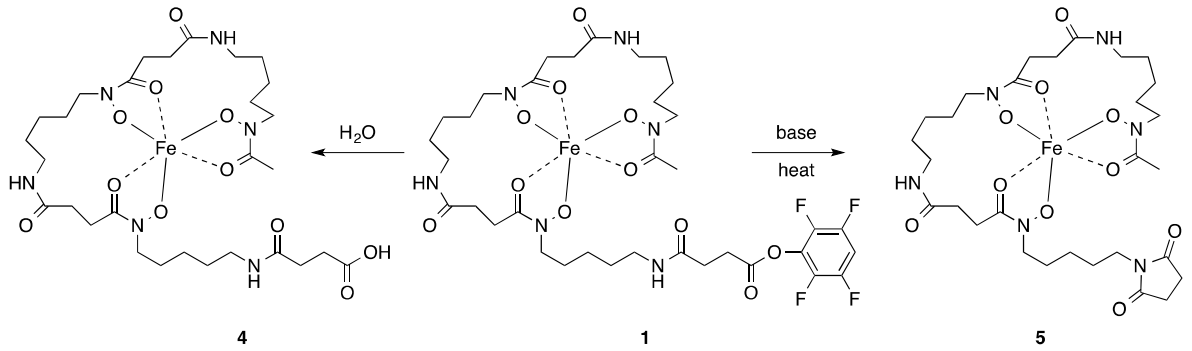
4 **1**

Entry	Reagent	Solvent	Time (h)	1(A% HPLC)[c]
1	EDC•HCl	NMP	12	45
2	EDC•HCl	DMF	12	87
3	T3P[b]	NMP	24	0
4	T3P[b]	DMF	24	0
5	CDI	NMP	24	4
6	CDI	DMF	24	3
7[d]	EDC•HCl	DMF	5	88

[a] Reaction performed with **4** (38 μmol, 100 mol%), coupling reagent (76 μmol, 200 mol%), 2,3,5,6-tetrafluorophenol (76 μmol, 200 mol%), *N*,*N*-diisopropylethylamine (76 μmol, 200 mol%) and 0.5 mL of solvent (20 mL/g). [b] Reaction performed with T3P (57 μmol, 150 mol%), 2,3,5,6-tetrafluorophenol (46 μmol, 120 mol%) and 4-methylmorpholine (57 μmol, 150 mol%). [c] Determined by HPLC analysis with UV detection at 254 nm. [d] Reaction performed with **4** (60.9 □mol, 100 mol%), EDC•HCl (122 mmol, 200 mol%), 2,3,5,6-tetrafluorophenol (122 mmol, 200 mol%), *N*,*N*-diisopropylethylamine (122 mmol, 200 mol%) and 400 mL of DMF (10 mL/g)

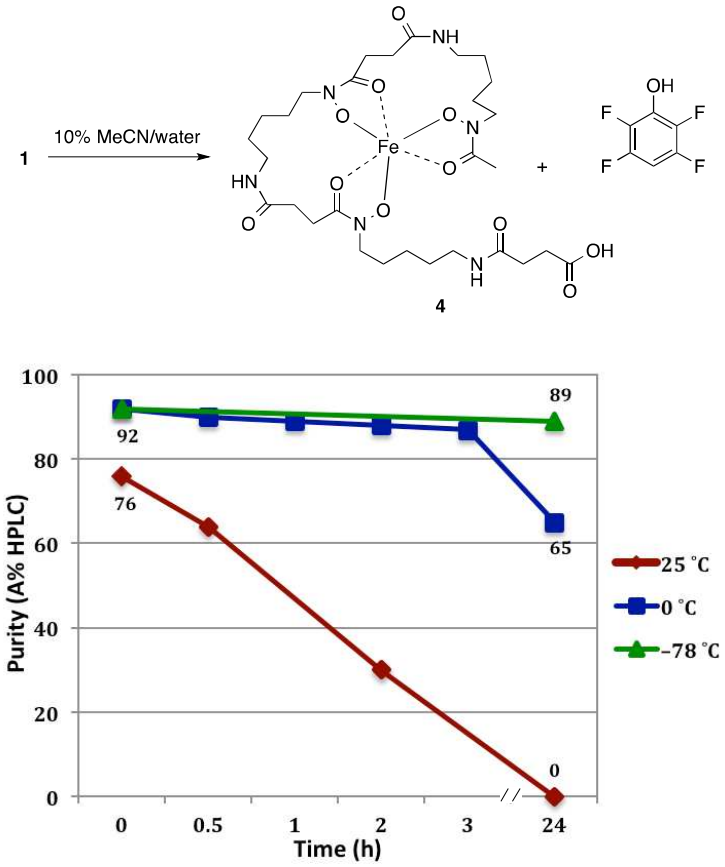
Work-up of the coupling reaction was accomplished by dilution with dichloromethane and washing with a 13% NaCl aqueous solution to remove the reaction solvent DMF and several reaction by-products such as succinic acid, DIPEA•MsOH, DIPEA•HCl and 1-ethyl-3-(3-dimethylaminopropyl)urea. Two reaction impurities, residual acid **4** and succinamide **5**[10] (Scheme 4) resulting from intramolecular displacement of the TFP leaving group, were also removed by these washes. The use of a 13% brine solution was necessary to achieve suitable phase separation during these washes as attempts at using pure water resulted in a large rag layer and high amounts of hydrolyzed product **4**.

Scheme 4. By-products Resulting from Attempted Recrystallization



All attempts to isolate active ester **1** via crystallization were unsuccessful and resulted in the isolation of degradation products (Scheme 4). The TFP ester **1** rapidly underwent hydrolysis to give **4** when exposed to adventitious water during solvent swapping procedures and when filtered, stability studies showed that this hydrolysis occurs rapidly at 25 ˚C (rate > 20%/h in a 70:30 mixture of acetonitrile/water, Figure 1). Upon exposure to base or extended heating in anhydrous solvent, compound **1** underwent an intramolecular cyclization reaction to give complete conversion to succinamide **5** (Scheme 4). Furthermore, no crystalline form for this material has been identified so the prospects of achieving a successful crystallization procedure were quite low.

Figure 1. Stability of Linker-Chelator 1 in 70:30 MeCN/water Eluent[a,b]

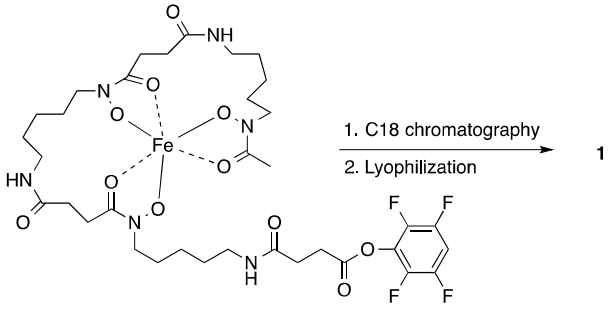


[a] Determined by HPLC analysis with UV detection at 254 nm. [b] Purity of fraction immediately after elution from C18 column is the fraction purity at t = 0.

As crystallization was not an option for isolation of the highly reactive linker-chelator **1**, we turned our attention to establishing a chromatographic means of isolation. After observing decomposition products with normal phase silica, amine-modified silica and alumina, a successful purification was obtained by using C18 reverse-phase silica.[11] A key observation was that while chromatography successfully removed a number of small impurities from the crude product, it also resulted in a significant amount of hydrolysis as a result of using water as the mobile phase. In light of these results, the chromatography procedure was modified in several key ways in an attempt to minimize hydrolysis. The starting ratio of the mobile phase was changed from 5% to 10% acetonitrile in water in order to reduce the amount of time the material spent on the column. Also towards this end, the continuous gradient was changed to a step gradient as this enabled high column loadings of up to 14.5%[12] using a

capture/release method rather than a true chromatographic separation. Most importantly, we also examined the amount of hydrolysis that took place in the collected fractions after elution. Not surprisingly, hydrolysis occurred very rapidly when the fractions were left at 25 ˚C with 12% degradation observed after only 30 minutes (Figure 1). Holding the fractions at 0 ˚C reduced the rate of hydrolysis significantly but did not eliminate it completely. Freezing the fractions in dry ice at –78 ˚C gave the best results with only minimal hydrolysis observed as the material purity decreased by only two percent from 91 A% HPLC to 89 A% HPLC over 24 hours. Implementing these improvements to the purification procedure resulted in a dramatic increase in final product purity (Table 3). Whereas the highest observed purity using the continuous gradient and not freezing the fractions was only 86 A% HPLC (entry 1), up to 92 A% HPLC purity was achieved using the step gradient and freezing the fractions before combining them for lyophilization (entry 3).[13]

Table 3. Optimization of the C18 Purification Procedure



1

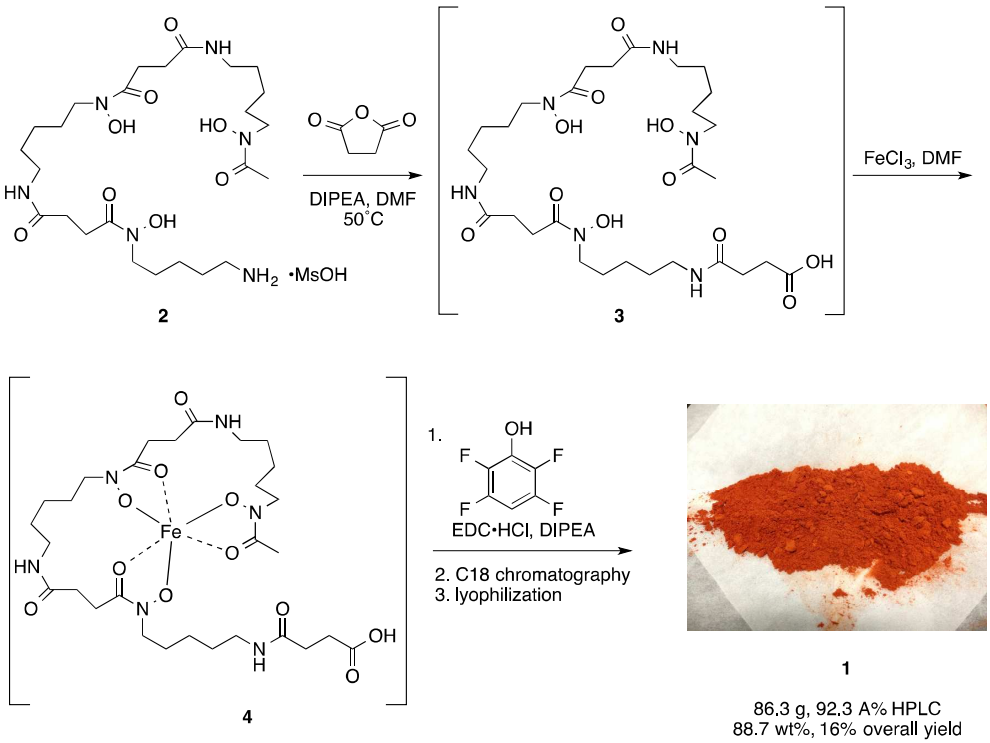
Entry	Scale (g)[a]	Gradient	Hold Temp (˚C)[b]	Yield(%)[c]	Purity[d]
1[b]	20.0	continuous	25	23	86
2	20.0	continuous	25	26	78
3	40.0	step	–78	14	92
4	40.0	step	–78	22	90

[a] Amount of amine starting material **2** taken through telescoped synthesis and work-up procedure prior to purification; [b] Fractions were sampled for HPLC analysis then either held at rt or frozen in a dry-ice acetone bath until column purification and analysis was complete; [c] Isolated yield after lyophilization; [d] Determined by HPLC analysis with UV detection at 254 nm.

Having developed a telescoped procedure for the linker-chelator synthesis and an efficient C18 reverse-phase purification procedure, we next embarked upon a campaign to deliver gram quantities of **GNE-605** for conjugation (Scheme 5). Our improved process was demonstrated on a 609 mmol, 400 g

scale of DFO mesylate to produce 100 mmol, 86.3 g of active ester **1** as a red solid with 92.3 A% HPLC purity and 88.7 wt% assay in 16% overall yield after chromatographic purification[14]. The only residual solvents detected were acetonitrile at 855 ppm and water at 1.5 wt%. Residual metals were also all within ICH limits (iron excluded).[15] To facilitate the ease of use of this material for conjugations, it was packaged under nitrogen into individual vials containing 1.2 g of the linker-chelator **1** and stored with dessicant at –20 ˚C to minimize hydrolysis. Subsequent stability studies showed that this material was stable for up to two years when stored under these conditions with 0% degradation based on HPLC analysis.

Scheme 5. Synthesis of GNE-605



CONCLUSION

In conclusion, a practical process for manufacturing the Immuno-PET linker-chelator **GNE-605 (1)** from commercially available desferrioxamine B on multi-gram scale has been developed. Modifications to the previously published synthetic procedure allow for an efficient 3-step telescoped process with significantly reduced reaction times, reduced raw material requirements and elimination of

most of the aqueous workup operations. A single straightforward work-up procedure to remove reaction by-products was introduced and helped to facilitate a subsequent C18 chromatographic purification. Significant optimization of the column purification minimized the amount of hydrolysis and significantly increased the purity of the isolated material to > 90 A% HPLC purity. For the first time, gram quantities of the Immuno-PET linker-chelator **GNE-605** have been synthesized and isolated with a high level of purity. This material has since been successfully conjugated to a number of therapeutic monoclonal antibodies and the small amount of material required for such conjugations means that this single campaign will enable the development of many future conjugates of interest.

EXPERIMENTAL SECTION

General: Solvents and reagents were obtained from commercial sources and used without further purification. DFO mesylate salt **2** was purchased from ECA with >93 A% HPLC purity. C18 chromatography was performed with a Thomson Single Step Pump® with manual fraction collection and a lyophilization was performed with a VirTis benchtop lyophilizer. Unless otherwise noted all reactions were run under a nitrogen atmosphere. Unless otherwise noted, ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) were recorded in DMSO-d_6. Chemical shifts are reported in ppm (δ units) downfield of internal tetramethylsilane [$(CH_3)_4Si$] or residual DMSO-d_6; coupling constants are reported in hertz (Hz). Multiplicities are as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet. Melting points and T_g are uncorrected and were measured by differential scanning calorimetry (DSC Q200 from TA Instrument). IR spectra were recorded on solid samples using Smart Orbit on a Thermo Nicolet 6700. UV spectra were recorded as solutions in MeCN.

HPLC Methods: HPLC analysis for compound **3** was performed by diluting the reaction mixture in 0.1M sodium carbonate (aq) with detection at 220nm (UV). HPLC analysis for compound **4** was performed by diluting the reaction mixture in 1:1 MeCN/water with detection at 420 nm (Vis). HPLC analysis for compound **1** was performed by diluting the reaction mixture in MeCN with detection at 254 nm (UV). HPLC method for monitoring the formation of **3**, **4** and **GNE-605(1)**: ACE 3 C18-HL, 3.0 x

50 mm, gradient elution from 95:5 to 5:95 0.05% TFA in water/ 0.05% TFA in MeCN over 4.8 min, 1.0 mL/min flow at 35 ˚C with detection at 220 nm, 254 nm and 420 nm. HPLC retention times: **2** = 2.309 min, **3** = 2.481 min, **4** = 2.289 min, **GNE-605** = 3.277 min.

Crude Iron (III), [2,3,5,6-tetrafluorophenyl-3,14,25-tri(hydroxy-κ*O*)-2,13,24-tri(oxo-κ*O*)-10,21,24-trioxo-3,9,14,20,25,31-hexaazapentatriacontan-35-oato(3-)]-(9CI) Coordination Compound (GNE-605): To a 20 L reactor under nitrogen was charged DMF (3.79 kg, 9.48 kg/kg), **2** (0.400 kg. 0.609 mol, 100 mol%), succinic anhydride (0.070 kg, 0.700 mol, 115 mol%), *N*,*N*-diisopropylethylamine (0.0787 kg, 0.609 mol, 100 mol%) and the mixture was heated at 50 °C for at least 1 h. The reaction was deemed complete when **2** ≤ 5.0 A% by HPLC at 220 nm. An analytically pure sample of **3** for characterization was isolated by vacuum filtration of the crude mixture and rinsing with DMF and diethyl ether. Compound **3** was obtained as a free flowing white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (1H, brs), 9.64-9.62 (3H, m), 7.79-7.75 (3H, m), 3.48-3.44 (6H, m), 3.03-2.98 (6H, m), 2.60-2.56 (4H, m), 2.43-2.40 (2H, m), 2.31-2.26 (6H, m), 1.97 (3H, s), 1.52-1.20 (18H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.31, 172.43, 171.77, 171.20, 170.61, 47.56, 47.26, 30.49, 30.39, 29.69, 29.27, 28.03, 26.49, 23.95, 20.80; mp = 163.85 ºC; IR (cm^{-1}) 3297.2, 2928.3, 2850.1, 1721.7, 1564.3, 1427.5, 1415.3, 1396.1, 1373.9, 1345.5, 1297.1, 1269.5, 1251.3, 1223.1, 1196.9, 1162.9, 1134.2, 1084.9, 1044.7, 968.3, 961.5, 933.0; HRMS (ESI+) calculated for $C_{29}H_{52}N_6O_{11}$ $[M+H]^+$, 661.3772 found 661.3779. The mixture was cooled to 25 °C and a solution of $FeCl_3$ (0.108 kg, 0.670 mol, 100 mol%) in DMF (0.342 kg, 0.855 kg/kg) was added. The reaction mixture was then agitated at 25 °C for a minimum of 30 min and deemed complete when **3** ≤ 5.0 A% by HPLC at 420 nm. An analytically pure sample of **4** could not be obtained as a solid as the compound was unstable during isolation and decomposed to a black tar. Solution phase characterization was obtained by filtering the crude to remove solids and analyzing the DMF solution: UV (nm) 202, 430; HRMS (ESI+) calculated for $C_{29}H_{49}FeN_6O_{11}$ $[M+H]^+$, 714.2887 found 714.2890. The mixture was charged with EDC•HCl (0.233 kg, 1.22 mol, 200 mol%), *N*,*N*-diisopropylethylamine (0.157 kg, 1.22 mol, 200 mol%) and a solution of 2,3,5,6-tetrafluorophenol

(0.111 kg, 0.670 mol, 110 mol%) in DMF (0.342 kg, 0.855 kg/kg). The mixture was agitated at 25 ˚C for a minimum of 5 h and monitored by HPLC until completion (**4** ≤ 10.0 A% by HPLC at 254 nm). The contents of the reactor were cooled to 5 ˚C and DCM (5.32 kg, 13.3 kg/kg) was charged. The mixture was warmed to 25 ˚C and charged with a 13% aqueous NaCl solution (13.2 kg, prepared from 4.52 kg NaCl and 34.8 kg H_2O). The mixture was agitated for a minimum of 1 min and the layers were separated. The organic phase was collected and dichloromethane (5.32 kg, 13.3 kg/kg) was charged to the reactor. The mixture was agitated for a minimum of 1 min then separated and the organic layer was collected and combined with the first organic phase. The combined organic phase was washed twice more with 13% aqueous NaCl (8.8 kg per wash) and recharged to the reactor. Na_2SO_4 (0.600 kg, 1.5 kg/kg) was charged and the resulting suspension was agitated for a minimum of 5 min. The slurry was filtered and the filtrate was concentrated under vacuum with a bath temperature of 25 ˚C. Dichloromethane (3.00 kg, 7 kg/kg) was charged to the reactor, and the resulting solution was filtered and concentrated under vacuum with a bath temperature of 25 ˚C to give crude **1** as a dark red, viscous oil (0.746 kg, 88.5 A% HPLC). The crude product was flushed with nitrogen, sealed and stored at 2–8 ˚C until it could be used in the purification.

General Procedure for Purification: A 413 g RediSep® gold column was conditioned with a 50% MeCN/water solution (2.2 L) with a StepPump motor setting of 42 (86 mL/min). The column was then equilibrated with a 10% MeCN/water solution (0.75 L) and loaded with crude **GNE-605** (56 mL). The column was eluted with 10% MeCN/solution at a StepPump motor setting of 25 (50 mL/min) until the eluent became visibly red and continued to be eluted until the eluent once again became colorless. All eluents up to this point were discarded. The column was eluted with a 30% MeCN/water solution at a StepPump motor setting of 35 (70 mL/min), and fractions of approximately 150 mL were collected in 250 mL glass bottles with screw tops. The fractions were labeled in sequential order, sampled for IPC analysis, capped securely and stored frozen in dry ice. In order to minimize hydrolysis, each fraction from the column was sampled and then frozen in dry ice until all the IPC data had been collected. To

ensure accurate IPC data, each IPC sample was itself diluted 10-fold with acetonitrile and frozen until it could be analyzed by HPLC. A total of 1.7 L of eluent was collected. The column was eluted with 100% MeCN until the eluent was no longer visibly colored and the eluent was discarded. The column was equilibrated with a 10% MeCN/water solution (0.75L) then loaded with another 55 mL of crude **GNE-605**. The overall process was repeated until a total of 11 column purifications had been performed. The fractions that passed IPC (**GNE-605** ≥ 85.0 A% HPLC at 254 nm) were thawed, combined and lyophilized to give **GNE-605** (86.3 g, 92.3 A% HPLC, KF: 1.5%, 16% yield). The material was packaged in vials containing approximately 1.2 g apiece. The vials were flushed with nitrogen, placed in LDPE bags with desiccant, sealed and stored at –20 ˚C. Compound **1** was obtained as a free-flowing bright red-orange powder, T_g = 108.7 ˚C; IR (cm^{-1}) 3295.7, 2927.4, 2857.9, 1786.4, 1644.0, 1572.8, 1522.3, 1486.4, 1453.6, 1356.1, 1260.5, 1217.5, 1177.7, 1104.0, 1066.2, 952.1, 841.6, 756.1; UV (nm) 198, 434; HRMS (ESI+) calculated for $C_{35}H_{49}F_4FeN_6O_{11}$ $[M+H]^+$, 862.2818, found 862.2811; Fe content (ICP-MS) calculated for $C_{35}H_{49}F_4FeN_6O_{11}$, 6.5%, found 7.3%. Because of the paramagnetic nature of the Fe(III) chelate, NMR spectroscopic data could not be obtained on **GNE-605**.

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SUPPORTING INFORMATION

Additional details on optimization of the purification procedure along with ^{1}H NMR, ^{13}C NMR, IR, DSC and UV spectra are available in the supporting information.

REFERENCES

[1] For reviews on Immuno-PET platforms and their use in the clinic see: (a) van Dongen, G. A. M. S.; Visser, G. W. M.; Lub-de Hooge, M. N.; de Vries, E. G.; Perk, L. R. *The Oncologist* **2007**, *12*, 1379. (b) Knowles, S. M.; Wu, A. M. *J. Clin. Onc.* **2012**, *30*, 2884.

[2] Verel, I; Visser, G. W. M.; Boellard, R.; Stigter-van Walsum, M.; Snow G. B.; van Dongen, G. A. M. S. *J. Nuc. Med.* **2003**, *44*, 1271.

[3] Also referred to in the literature as desferoxamine, DFO-B, DFOA, DFB, desferal and DFO

[4] Tenenbein, M. *J. Toxicol. Clin. Toxicol.* **1996**, *34*, 485.

[5] Solubility of compound **3** in 0.15 M NaOH is < 1 mg/mL.

[6] In reference 2, the amount of compound **1** produced is estimated based on an unquantified HPLC trace

[7] See experimental section, HPLC methods.

[8] Further optimization showed that these reagents could be further reduced to 1.8 molar equivalents and 1.1 equivalents respectively.

[9] Attempts were also made to accomplish the esterification on the non-chelated linker **3** with a number of solvents (NMP, DMF, THF, MeCN, DMSO) and reagents (EDC•HCl, T3P, CDI). However, in all cases no reaction was observed as chelation of the hydroxyamides appears to be a necessary condition for successful functionalization of the terminal acid.

[10] Residual acid **3** was observed at up to 10 A% by HPLC and succinimide **5** was observed at up to 5 A% by HPLC using the standard HPLC method described in the experimental section.

[11] Several brands of columns were compared for their purification effectiveness and recovery efficiency with the RediSep® Gold brand column giving the highest level of purity and material recovery. See Supporting Information for further details.

[12] See experimental for details. Up to 60 g of crude **1** was loaded onto a 413 g C18 column.

[13] For lyophilization it is important to maintain sufficiently strong vacuum (< 1000 mtorr) in order to avoid melt-back. In cases where vacuum was not sufficiently strong, dark black, glasslike particles consisting of the hydrolysis product **4** would form on the surfaces where melt-back had occurred.

[14] Crude ester **1** was stored at 2–8 ˚C under a nitrogen atmosphere in an attempt to minimize hydrolysis while awaiting column purification. Even so, after 24 hours of storage the purity of the crude material was found to have degraded down to 87 A% purity from 88 A% purity and degradation continued at a rate of approximately 1% every 24 hours with 77 A% HPLC purity after 8 days of storage.

[15] Cd < 5 ppm; Pb < 10 ppm; As < 3ppm; Hg < 3 ppm.