O-Protected *N*-(2-Nitrophenylsulfonyl)hydroxylamines: Novel Reagents for the Synthesis of Hydroxamates

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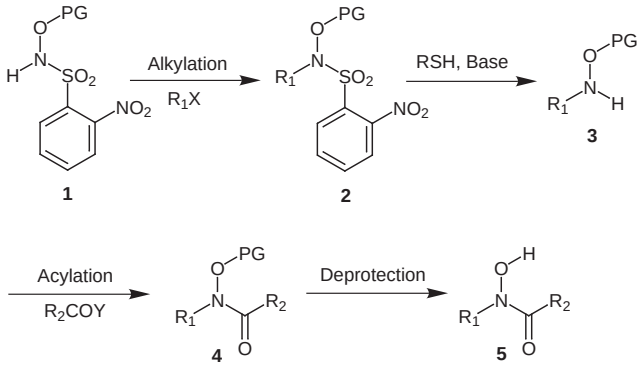
Abstract: Preparative methods for novel *O*-protected *N*-(2-nitrophenylsulfonyl)hydroxylamines (**8a–e**) are described. Their versatility as intermediates en route to polyhydroxamates is exemplified by the synthesis of a non-amide DFO analog **22**.

Key words: hydroxamate, nosyl protecting group, solid-phase synthesis, alkylation, Mitsunobu reaction

The naturally occurring siderophore Desferrioxamine B (Desferal, DFO),[1] which contains three hydroxamate groups that form a hexadentate-complex with ferric ion, has been used for the treatment of iron overload for many years, and still remains the drug of choice.[2] The shortcomings of this chelating therapy, mainly poor compliance of patients associated with subcutaneous infusion of the drug, necessitates the development of a new iron chelator that is orally active and safe. The solution-phase syntheses of several important siderophores, including DFO[3,4] and analogs,[5,6] have been accomplished. The solution synthetic methods used, however, are not suitable for generating large numbers of structurally diverse hydroxamate analogs.

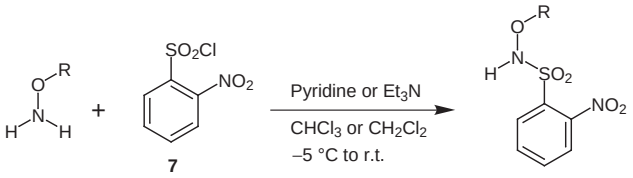
In order to facilitate structure–activity studies on naturally occurring hydroxamates, such as DFO, and pursue chemical diversity with novel structures capable of binding iron, we have developed a flexible synthetic strategy involving *N*,*O*-bisprotected hydroxylamine **1** as the key intermediate (Scheme 1). The presence of the temporary *N*-protecting 2-nitrophenylsulfonyl (nosyl) group activates the nitrogen for alkylation with alkyl halides by conventional methods or alcohols under Mitsunobu reaction conditions to give **2**. Selective removal of the nosyl group with thiolate anion then leads to **3** that facilitates further elaboration of the molecule by *N*-acylation to afford the intermediate **4**. Removal of the permanent *O*-protecting group (PG) leads to the desired hydroxamate **5**. This approach is very versatile and is suitable for both solid- and solution-phase methods.



R = alkyl or aryl
R_1 = alkyl, arylalkyl, or appropriately derivatized polystyrene-based resin
R_2 = alkyl, aryl, or arylalkyl

Scheme 1

In this context, we wish to report the synthesis and characterization of novel *O*-protected *N*-(2-nosyl)hydroxylamines, **8a–e** (Scheme 2). The choice of 2-nosyl group for *N*-protection is based on the reported utility of *o*-and *p*-nitrophenylsulfonamides in selective *N*-alkylation of primary amines[7,8] and esters of α-amino-acids,[9,10] as well as the generation of *N*-alkyl peptides on the solid support.[11–13] In addition, the thiolate-labile nosyl group is orthogonal not only to the *O*-protecting groups utilized here, but also to most linkers to the solid support. The choice of *O*-protecting groups,[14] particularly, acid-labile *t*-Bu, THP, and 2,4-dimethoxybenzyl[15] makes the subsequent automated high-throughput solid-phase synthesis a more robust process, as the protecting group can also be removed



6a: R = Bn
6b: R = allyl
6c: R = *t*-Bu
6d: R = THP
6e: R = 2,4-$(MeO)_2$Bn

8a: R = Bn (70%)
8b: R = allyl (51%)
8c: R = *t*-Bu (80%)
8d: R = THP (79%)
8e: R = 2,4-$(MeO)_2$Bn (54%)

Scheme 2

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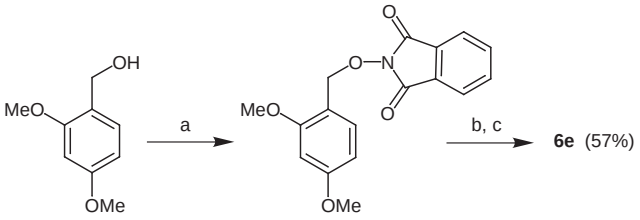
during the final cleavage from the solid support. Some of the known *N*,*O*-bisprotected hydroxylamine derivatives used for the synthesis of hydroxamates, including BOC-NH-OTHP,[16] BOC-NH-OTBDMS,[16] BOC-NH-OBn,[3,6] and *O*-(2,4)-dimethoxybenzyl-*N*-(2,4,6)-trimethoxybenzylhydroxylamine,[15] and certain previously reported N-arylsulfonyl-*O*-substituted hydroxylamine derivatives,[17,18] suffer mainly from a lack of orthogonality of the *N*- and *O*-protecting groups during deprotection.

To this end, commercially available *O*-benzylhydroxylamine (**6a**) hydrochloride was reacted with 2-nosyl chloride (**7**, 0.8 or 1.5 equivalents) in CH_2Cl_2 in the presence of *N*,*N*-diisopropylethylamine (DIPEA) (2.6 or 4.0 equivalents) for 2 hours at room temperature. This approach failed to give a reasonable yield of the desired product **8a**. The acidic NH group in the *O*-protected hydroxamate (pKa ~10) is susceptible to further sulfonylation in the presence of strongly basic DIPEA (pKa ~11), and in fact, the major product isolated in the reaction was the corresponding disubstituted derivative.[19]

Consequently, the reaction was carried out in pyridine (pKa = 5.23) with stoichiometric amounts of the reagents for 30 minutes at –5 °C and then for 2 hours at room temperature, and the desired product **8a** was obtained in good yield (70 %). A similar reaction of **6b** with **7** (1.5 equivalents) in pyridine gave **8b** in 51% yield. Similarly, commercially available *O*-*tert*-butyllhydroxylamine (**6c**) hydrochloride was treated with **7** (1 equivalent) in the presence of Et_3N (2.1 equivalents) as the base in $CHCl_3$ for 2 hours at –5 °C, and then overnight at room temperature to afford **8c** in 80% yield. No dinosyl-substituted byproduct was isolated, probably due to the steric bulk of the adjacent *tert*-butyl group. The reaction was slightly modified for the preparation of THP derivative **8d**, wherein pyridine and CH_2Cl_2 were substituted for Et_3N and $CHCl_3$, respectively. Thus, the reaction of stoichiometric amounts of **6d**[20] and **7** in the presence of pyridine (1.5 equivalents) in CH_2Cl_2 for 2 hours at –5 °C, and then overnight at room temperature, gave **8d** in 79% yield. A similar reaction of **6e**[15] with **7** (1.1 equivalents) in the presence of pyridine (2.0 equivalents) in CH_2Cl_2 gave **8e** in 54% yield. It should be mentioned that 4-nitro-analogs of **8a** and **8c** were also prepared by reacting **6a** and **6c** with stoichiometric amounts of 4-nosyl chloride in pyridine (–5 °C, 2 hours and room temperature, 2 hours; 87%) and in the presence of Et_3N (2.1 equivalents) in $CHCl_3$ as the solvent (–5 °C, 2 hours and room temperature, 3 hours; 59%), respectively.[21]

Based on our initial observation that removal of the 4-nosyl group demanded longer reaction times than that required for the corresponding 2-nosyl group, we have not explored the use of these compounds in solid-phase syntheses. Reports of minor byproduct formation during the alkylation of 4-nitrophenylsulfonamides of amino esters with less reactive alkylating reagents (intramolecular transfer of the nitrophenyl group to the α-carbon of the amino ester),[9,22] and the poor regioselectivity observed during denosylation of some 4-nitrophenylsulfonamides (displacement of nitro group by thiolate rather than sulfonamide cleavage)[23] prompted us to discontinue the use of 4-substituted analogs.

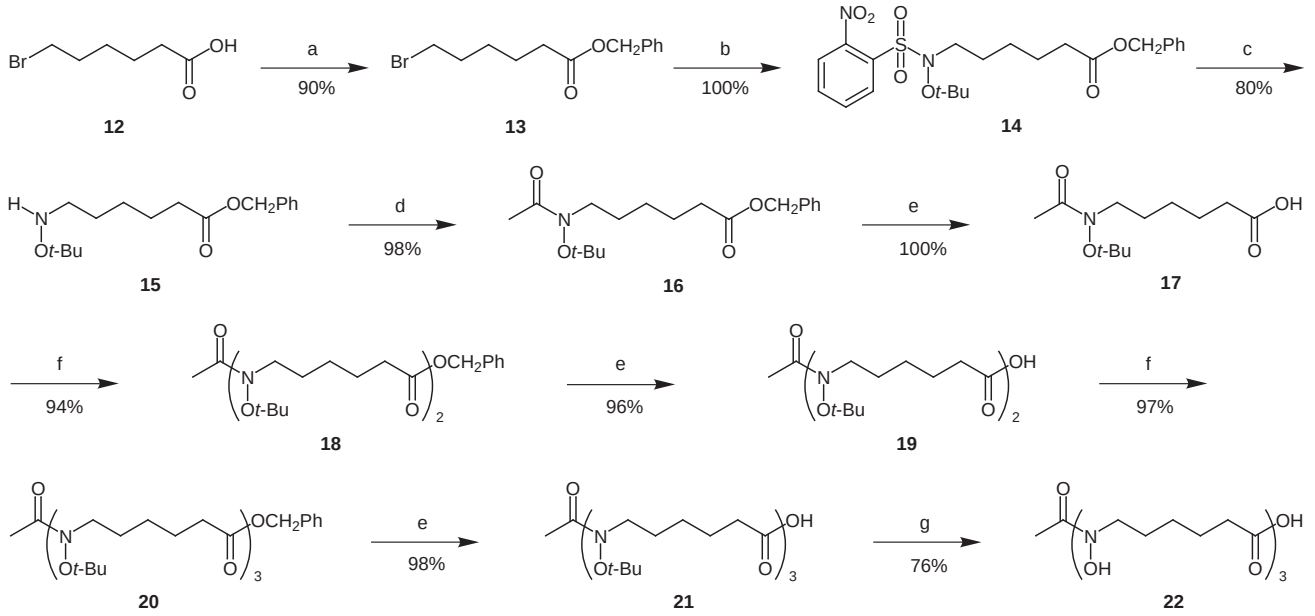
During the above synthesis of **8e**, the hydroxylamine derivative **6e** was prepared in 76% yield by reacting 2,4-dimethoxybenzylalcohol (**9**) and *N*-hydroxyphthalimide (**10**) under Mitsunobu conditions [Ph_3P, diethyl azodicarboxylate (DEAD), $CHCl_3$, room temperature, 17 hours], followed by hydrazinolysis with hydrazine in EtOH (reflux, 30 minutes) according to a reported method.[15] However, this involved extensive chromatographic purification of the intermediate phthalimide derivative **11** from byproducts, particularly, copious amounts of Ph_3PO. Additionally, after the hydrazinolysis step, compound **6e** could not be obtained entirely free of the corresponding byproduct phthalhydrazide. In an attempt to avoid this chromatographic step, we first carried out the Mitsunobu reaction in CH_2Cl_2; it was then feasible to wash the excess **10** with alkali solution. Subsequent hydrazinolysis was effected with methylhydrazine in toluene at 80 °C for 2 hours (Scheme 3), as described in the synthesis of **6d**.[20] The insoluble byproduct *N*-methylpthalhydrazide was separated by filtration and the neutral Ph_3PO carried over from the Mitsunobu reaction was separated by chemical purification (acid/base work up). The crude *O*-protected hydroxylamine **6e** (57% yield), thus obtained, was reacted with 2-nosyl chloride (**7**) in CH_2Cl_2 in the presence of pyridine as described above to give the product **8e** in 49% yield (overall 28% versus 41% in the previous method).



9 **11**

Scheme 3 Reagents and conditions: (a) *N*-Hydroxyphthalimide (**10**), Ph_3P, DEAD, CH_2Cl_2, r.t., 18 h; (b) $MeNHNH_2$, PhMe, 80 °C, 2 h; (c) chemical purification

An example highlighting the utility of nosylhydroxylamine derivatives of type **8** in the synthesis of hydroxamates is shown in Scheme 4. A non-amide analog of DFO, **22**, was synthesized by solution methods using *tert*-butyl as the *O*-protecting group for nosylhydroxylamine. Thus, 6-bromohexanoic acid (**12**) was esterified with benzyl alcohol, and the resulting known benzyl ester **13**[24] was employed in the *N*-alkylation of *O*-*tert*-butyl-*N*-nosylhydroxylamine (**8c**) in the presence of Cs_2CO_3, leading to **14** in quantitative yield. Deprotection of the nosyl group in **14** with dilithio-mercaptoacetic acid furnished one of the desired intermediates **15**. Treatment of **15** with acetic anhydride/pyridine followed by saponification of the benzyl ester supplied the other key intermediate **17**.



Scheme 4 Reagents and conditions: (a) $PhCH_2OH$, DIC, DMAP, THF, r.t., 4 h; (b) *t*-BuONHNs (**8c**), Cs_2CO_3, CH_3CN, reflux, 6 h; (c) $HSCH_2CO_2H$, LiOH, DMF, r.t., 2.5 h; (d) Ac_2O, pyridine, r.t., 3 h; (e) 1 N NaOH, EtOH, r.t.; (f) **15**, HATU, DIPEA, DMF, r.t., 2 h; (g) TFA, r.t., 10 h

The coupling of the *N*,*O*-bisprotected carboxylic acid **17** to the hydroxylamine moiety of the intermediate **15** was achieved with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) in DMF leading to the dimeric ester **18** in high yield. Subsequent hydrolysis of the ester followed by coupling once again with **15** furnished trimeric ester **20**. After saponification, the *tert*-butyl groups of the trimeric acid **21** were deprotected with TFA under anhydrous conditions, to give the target trihydroxamate **22** in good overall yield (46%). Although the example shown here is based on solution chemistry, our primary focus is solid-phase synthesis. In fact, we have generated several polyhydroxamate libraries (manuscripts in preparation) by solid-phase syntheses.[25]

In summary, we have developed methods for the preparation of O-substituted *N*-(2-nitrophenylsulfonyl)hydroxylamines **8a–e** that are useful intermediates in the synthesis of polyhydroxamates.

Unless otherwise noted, all reagents were used as supplied. Organic extracts were dried either with Na_2SO_4 or $MgSO_4$ and filtered. Solvents were removed on a rotary evaporator under reduced pressure. Analytical TLC was performed on Analtech silica gel GF plates (250 μm). Flash chromatography was carried out using Fisher Scientific silica gel 60A (38–90 μm). All mps were determined on a Thomas-Hoover (Model 67T108) capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian Gemini-300 spectrometer. Chemical shifts (δ) are expressed in ppm relative to TMS as an internal standard. Coupling constants (*J*) are reported in Hz. Electrospray mass spectra were obtained on a ThermoQuest AQA spectrometer. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA.

***N*-(2–Nitrophenylsulfonyl)-*O*-(phenylmethyl)hydroxylamine (8a)**

A 250-mL round-bottom flask fitted with an addition funnel was charged with *O*–benzylhydroxylamine (**6a**) hydrochloride (5.00 g, 31.3 mmol) and the solid was partially dissolved in anhyd pyridine (60 mL) by stirring under a flow of N_2. The flask was immersed in an ice-salt water bath and cooled to about –5 ºC. A greenish brown solution of 2-nitrobenzenesulfonyl chloride (**7**, 7.1 g, 32 mmol) in anhyd pyridine (20 mL) was added dropwise at a rate of ~1 drop per second, while the temperature was maintained at –5 °C throughout the addition. Following the addition, the brownish orange solution was stirred for 30 min more at this temperature, then allowed to warm to r.t. and stirred for a total of 2 h. H_2O (15 mL) was added to terminate the reaction and afford a clear solution, and the solvents were removed. The resulting dark amber syrup was taken up in EtOAc–H_2O (1:1, 400 mL) and partitioned in a separatory funnel. The organic layer was washed successively with 5% HCl, H_2O, and sat. $NaHCO_3$ (200 mL each). Solvent removal gave a brownish orange solid, which was dissolved in boiling EtOH–H_2O (9:1, 200 mL), treated with charcoal (2 g), and filtered. The filtrate was reheated and allowed to cool to r.t., depositing small-to medium-sized light yellow rhombic crystals of the product **8a** (6.7 g, 70%). Homogeneous by TLC. $R_f = 0.52$ (EtOAc–hexanes, 3:7).

Mp 149–150 °C.

^{1}H NMR ($CDCl_3$): δ = 8.27–8.23 (m, 1H, NsH-3), 8.12 (s, 1H, NH), 7.90–7.76 (m, 3H, NsH-4, 5, 6), 7.37 (s, 5H, BnH), 5.07 (s, 2H, OCH_2Ph).

^{13}C NMR (DMSO-d_6): δ = 148.91, 135.58, 135.29, 132.68, 130.22, 129.62, 129.25, 128.63, 128.54, 124.62, 78.69.

ESI MS: *m/z* = 309 $(M + H)^+$, 326 $(M + NH_4)^+$.

Anal. Calcd for $C_{13}H_{12}N_2O_5S$: C, 50.65; H, 3.92; N, 9.09; S, 10.40. Found: C, 50.51; H, 3.91; N, 8.99; S, 10.53.

***N*-(2–Nitrophenylsulfonyl)-*O*-(2-propenyl)hydroxylamine (8b)**

The reaction of *O*–allylhydroxylamine (**6b**) hydrochloride (3.00 g, 27.4 mmol) with 2-nitrobenzenesulfonyl chloride (**7**, 9.1 g, 41 mmol) in anhyd pyridine (50 mL) was carried out as described

above in the preparation of **8a**. After the usual work up, the crude product was isolated as a dark brown solid. It was dissolved in boiling EtOH–H_2O (9:1, 100 mL), treated with charcoal (1 g), and filtered. The filtrate was reheated and allowed to cool in a refrigerator overnight, whereby light orange flakes of the product **8b** (3.5 g, 51%) were separated. Homogeneous by TLC. $R_f = 0.35$ (EtOAc–hexanes, 3:7).

Mp 110–111 °C.

^{1}H NMR ($CDCl_3$): δ = 8.26–8.20 (m, 1H, NsH-3), 8.12 (s, 1H, NH), 7.94–7.78 (m, 3H, NsH-4, 5, 6), 6.00–5.86 (m, 1H, C*H*=CH_2), 5.38–5.29 (m, 2H, CH=C*H*$_2$), 4.56 (d, 2H, *J* = 6.3, OCH_2).

^{13}C NMR ($CDCl_3$): δ = 148.74, 135.07, 133.96, 133.11, 131.91, 130.45, 125.76, 120.82, 78.66.

ESI MS: *m/z* = 259 $(M + H)^+$, 276 $(M + NH_4)^+$.

Anal. Calcd for $C_9H_{10}N_2O_5S$: C, 41.86; H, 3.90; N, 10.85; S, 12.41. Found: C, 41.86; N, 3.97; N, 10.68; S, 12.43.

O-(1,1-Dimethylethyl)-*N*-(2-nitrophenysulfonyl)hydroxylamine (8c)

A 1-L round bottom flask fitted with an addition funnel was charged with *O*-*tert*-butylhydroxylamine (**6c**) hydrochloride (25.3 g, 201 mmol) and the solid was dissolved in anhyd $CHCl_3$ (400 mL) by stirring under N_2. The flask was immersed in an ice-salt water bath, cooled to about –5 °C and Et_3N (58.8 mL, 422 mmol) added dropwise (approx. 15 min). Then a solution of 2-nitrobenzenesulfonyl chloride (**7**, 44.64g, 201.0 mmol) in anhyd $CHCl_3$ (250 mL) was added slowly (approx. 1.25 h), and the resulting brownish orange solution was stirred for 2 h at –5 °C, allowed to warm to r.t., and stirred overnight. The contents were transferred to a 2-L separatory funnel and washed successively with H_2O, 1 N HCl (× 2), H_2O, 5% $NaHCO_3$ (× 2), H_2O, and brine (200 mL each). Solvent removal gave 50.98 g of the crude product, which was recrystallized from EtOH (550 mL) to afford the product **8c** (44.26 g, 80%) as a colorless crystalline solid. Homogeneous by TLC. $R_f = 0.40$ (CH_2Cl_2–hexanes, 7:3).

Mp 149–150 °C.

^{1}H NMR ($CDCl_3$): δ = 8.19–8.15 (m, 1H, NsH-3), 7.90–7.75 (m, 3H, NsH-4, 5, 6), 7.61 (s, 1H, NH) 1.28 (s, 9H, CMe_3).

^{13}C NMR ($CDCl_3$): δ = 148.76, 134.76, 133.96, 132.88, 130.91, 125.54, 83.00, 26.74.

ESI MS: *m/z* = 275 $(M + H)^+$, 292 $(M + NH_4)^+$.

Anal. Calcd for $C_{10}H_{14}N_2O_5S$: C, 43.79; H, 5.14; N, 10.21; S, 11.69. Found: C, 43.96; H, 5.11; N, 10.10; S, 11.79.

N-(2-Nitrophenylsulfonyl)-*O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (8d)

To a solution of *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (**6d**, 101.0 mmol, 11.82 g),[20] and pyridine (151.5 mmol, 12.2 mL) in anhyd CH_2Cl_2 (275 mL), was added slowly (approx. 1 h) a solution of 2-nitrobenzenesulfonyl chloride (**7**, 101.0 mmol, 22.42 g) in anhyd CH_2Cl_2 (125 mL) containing pyridine (50.5 mmol, 4.1 mL) with stirring at –5 °C under N_2. The cooling bath was removed after 2 h, and the yellow solution was stirred overnight (approx. 14 h) at r.t. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with 5% $NaHCO_3$ (3 × 200 mL), and the solvent was removed to give 35.87 g of a dark viscous residue. Quick filtration through a bed of silica gel (100 g) packed in a fritted glass funnel using CH_2Cl_2–hexanes (4:1), followed by trituration of the concentrate with Et_2O–hexanes (1:3), afforded 26.01 g of slightly impure product as an off-white powder. Further purification was accomplished by flash chromatography over silica gel (300 g) using EtOAc–hexanes (2:3) containing Et_3N (0.5%) to give **8d** (24.14 g, 79%) as nearly colorless powder.[26] Homogeneous by TLC. $R_f = 0.49$ (EtOAc–hexanes, 1:1).

Mp 108–109 °C.

^{1}H NMR ($CDCl_3$): δ = 8.23−8.17 (m, 1H, NsH-3), 7.96−7.78 (m, 3H, NsH-4, 5, 6), 5.22 (t, 1H, *J* = 3.2, OCH), 3.91−3.83 (m, 1H, OCH_2), 3.70−3.60 (m, 1H, OCH_2), 1.82−1.50 [m, 6H, $(CH_2)_3$].

^{13}C NMR ($CDCl_3$): δ = 148.80, 135.10, 133.73, 133.09, 130.62, 125.82, 103.88, 63.01, 28.36, 24.89, 18.86.

ESI MS: *m/z* = 325 $(M + Na)^+$, 301 $(M - H)^-$.

Anal. Calcd for $C_{11}H_{14}N_2O_6S$: C, 43.70; H, 4.67; N, 9.27; S, 10.61. Found: C, 43.76; H, 4.68; N, 9.22; S, 10.73.

O-[(2,4-Dimethoxyphenyl)methyl]-*N*-(2-nitrophenylsulfonyl)-hydroxylamine (8e) Method A:

To a solution of *O*-2,4-dimethoxybenzylhydroxylamine (**6e**, 12.1 g, 66.0 mmol)[15] and pyridine (8.0 mL, 99 mmol) in anhyd CH_2Cl_2 (180 mL), was added slowly (approx. 1 h) a solution of 2-nitrobenzenesulfonyl chloride (**7**, 16.1 g, 72.6 mmol) in anhyd CH_2Cl_2 (85 mL) containing pyridine (2.66 mL, 33.0 mmol) with stirring at –5 °C under N_2. The resulting orange solution was slowly warmed to r.t. (approx. 4 h) and stirred overnight (approx. 14 h). The dark colored reaction mixture was successively washed with H_2O, ice-cold 1 N HCl, 5% $NaHCO_3$, H_2O, and brine (150 mL each). Removal of the solvent gave 24.3 g of an orange viscous residue, which was dissolved in boiling CH_2Cl_2 (50 mL) with stirring, and then boiling EtOH (200 mL) was added slowly. Upon cooling to r.t., the solution was seeded with a crystal of pure product and left overnight, whereupon product **8e** (8.21 g) was separated as a pale yellow crystalline solid. The filtrate was concentrated subjected to flash chromatography over silica gel (120 g) using CH_2Cl_2−hexanes (3:2) to obtain an additional 4.91 g of the product **8e** as a pale yellow foam, bringing the overall yield to 13.12 g (54%). Homogeneous by TLC. $R_f = 0.58$ (CH_2Cl_2−hexanes, 4:1).

Mp 124–125 °C.

^{1}H NMR ($CDCl_3$): δ = 8.27−8.22 (m, 1H, NsH-3), 8.02 (br s, 1H, NH), 7.87−7.73 (m, 3H, NsH-4, 5, 6), 7.28 (d, 1H, *J* = 8.2, BnH-6), 6.49−6.44 (m, 2H, BnH-3 & 5), 5.07 (s, 2H, OCH_2Ph), 3.82 (s, 3H, OMe), 3.81 (s, 3H, OMe).

^{13}C NMR ($CDCl_3$): δ = 162.17, 159.73, 148.69, 134.81, 134.02, 133.44, 132.96, 130.71, 125.60, 115.79, 104.29, 98.70, 74.73, 55.57, 55.50.

ESI MS: *m/z* = 369 $(M + H)^+$, 386 $(M + NH_4)^+$, 391 $(M + Na)^+$.

Anal. Calcd for $C_{15}H_{16}N_2O_7S$: C, 48.91; H, 4.38; N, 7.60; S, 8.70. Found: C, 48.98; H, 4.39; N, 7.55; S, 8.82.

Method B:

A 1-L round bottom flask equipped with an addition funnel was charged with 2,4-dimethoxybenzyl alcohol (**9**, 10.09 g, 60.00 mmol), *N*-hydroxyphthalimide (**10**, 12.72 g, 78.00 mmol), Ph_3P (20.46 g, 78.00 mmol), and anhyd CH_2Cl_2 (200 mL), and the suspension was stirred vigorously for 15 min at r.t. under N_2. Then an orange solution of DEAD (12.28 mL, 78.00 mmol) in anhydrous CH_2Cl_2 (40 mL) was added slowly (approx. 35 min) and stirred overnight (approx. 20 h) at r.t. The clear yellow reaction mixture was washed with 1 N NaOH (× 2), H_2O (× 2), and brine (100 mL each). Removal of the solvent gave crude *N*-[(2,4-dimethoxyphenyl)methyl]phthalimide (**11**, 43.91 g) as a thick yellow semi-solid, which was dried under high vacuum overnight.

The above crude phthalimide **11** (60.0 mmol) was co-evaporated with toluene (100 mL) and then additional toluene (220 mL) was added. The suspension was heated to 80 °C, and after dissolution of the solid, methylhydrazine (3.51 mL, 66.0 mmol) was added and the reaction mixture was stirred at that temperature for 2 h. After cool-

ing in an ice bath, the contents were filtered through a fritted glass funnel. The filtrate was chilled to near 0 °C in an ice bath and washed with ice-cold 2 N HCl (4 × 7 5 mL). The combined aqueous layers were washed with CH_2Cl_2 (2 × 100 mL), chilled to near 0 °C, and made alkaline by the slow addition of 3 N NaOH (solution becomes foggy-white upon becoming alkaline). The product was extracted with CH_2Cl_2 (4 × 125 mL),[27] and the combined CH_2Cl_2 extract washed successively with H_2O and brine (100 mL each). Solvent removal gave a clear amber-colored oil, which was dried under high vacuum to give *O*-[(2,4-dimethoxyphenyl)methyl]hydroxylamine (**6e**, 6.23 g, 57% for two steps) [ESI MS: *m/z* = 184 $(M + H)^+$, 167 $(M - NH_3)^+$].

The above crude hydroxylamine derivative **6e** (6.23 g, 34.0 mmol) was co-evaporated with anhyd CH_2Cl_2 (50 mL) and the residue reacted with 2-nitrobenzenesulfonyl chloride (**7**, 9.04 g, 40.8 mmol) in anhyd CH_2Cl_2 (135 mL) in the presence of pyridine (6.60 mL, 81.6 mmol) as base as described above in Method A. After the usual work up, the crude product (13.25 g) was treated with charcoal in a minimum amount of boiling CH_2Cl_2–EtOH (1:4). The filtrate was concentrated and passed through a short-path silica gel (15 g) column using CH_2Cl_2 (250 mL). Solvent removal followed by recrystallization from CH_2Cl_2–EtOH (1:4) afforded **8e** (5.88 g) as yellow crystals. The filtrate was concentrated and subjected to flash chromatography over silica gel (60 g) using CH_2Cl_2 to give 0.31 g of additional pure product **8e**, bringing the total yield to 6.19 g (49%, overall 28% from **9**).

6-Bromohexanoic Acid Benzyl Ester (13)

A slightly turbid solution of 6-bromohexanoic acid (**12**, 4.33 g, 22.2 mmol) and DMAP (0.32 g, 2.60 mmol) in THF (40 mL) was stirred and cooled to 0 °C, and treated with benzyl alcohol (1.90 mL, 18.5 mmol) followed by DIC (3.50 mL, 22.2 mmol) in THF (10 mL) under N_2. After 30 min at 0 °C, the cooling bath was removed and the reaction mixture was allowed to warm to r.t (approx. 15 min) and then stirred for 4 h. The reaction was quenched by the addition of H_2O (10 mL) and the solvent removed. The remaining light oil and white crystals were taken up in EtOAc–H_2O (100 mL each) and extracted with sat. $NaHCO_3$. Removal of solvent followed by flash chromatography over silica gel (EtOAc–hexanes, 1:9) afforded **13** (4.75 g, 90%) as a colorless oil.

^{1}H NMR ($CDCl_3$): Consistent with the reported data.[24]

ESI MS: *m/z* = 285 $(M + H)^+$, 302 $(M + NH_4)^+$, 307 $(M + Na)^+$.

6-[*N*-*tert*-Butyloxy-*N*-(2-nitrophenylsulfonyl)]aminohexanoic Acid Benzyl Ester (14)

An orange solution of hydroxylamine derivative **8c** (2.74 g, 10.0 mmol) and ester **13** (3.08 g, 10.8 mmol) in anhyd CH_3CN (50 mL), containing Cs_2CO_3 (6.52 g, 20.0 mmol) in suspension, was stirred and refluxed for 6 h under N_2. After cooling to r.t., Cs_2CO_3 was filtered off and the filtrate, which included acetone washings (25 mL), was concentrated to give 5.13 g of dark brown viscous residue. Flash chromatography over silica gel (30 g) (CH_2Cl_2) yielded **14** (4.79 g, quantitative) as a highly viscous pale yellow residue.

^{1}H NMR ($CDCl_3$): δ = 8.07 (dd, 1H, *J* = 7.8, 1.7, NsH-3), 7.78–7.66 (m, 2H, NsH-4, 5), 7.53 (dd, 1H, *J* = 7.8, 1.7, NsH-6), 7.37–7.34 (m, 5H, BnH), 5.11 (s, 2H, OCH_2Ph), 3.39 (br m, 1H, NCH_2), 3.05 (br m, 1H, NCH_2) (unresolved 'AB' pair), 2.34 (t, 2H, *J* = 7.4 Hz, CH_2CO), 1.80–1.30 [m, 6H, $(CH_2)_3$], 1.31 (s, 9H, CMe_3).

^{13}C NMR ($CDCl_3$): δ = 173.63, 149.73, 136.28, 134.75, 133.14, 131.03, 128.80, 128.43, 127.51, 123.62, 84.01, 66.27, 55.97, 34.10, 27.79, 26.94, 26.47, 24.54.

ESI MS: *m/z* = 496 $(M + NH_4)^+$.

Anal. Calcd for $C_{23}H_{30}N_2O_7S$: C, 57.73; H, 6.32; N, 5.85; S, 6.70. Found: C, 57.84; H, 6.51; N, 5.84; S, 6.83.

6-(*N*-tert-Butyloxy)aminohexanoic Acid Benzyl Ester (15)

To a suspension of $LiOH·H_2O$ (1.93 g, 46.0 mmol) in DMF (30 mL), mercaptoacetic acid (1.92 mL, 27.6 mmol) was added with stirring at r.t. under N_2. After the reaction mixture became homogeneous (approx. 15 min), a solution of **14** (5.50 g, 11.5 mmol) in DMF (10 mL) was added dropwise (approx. 10 min), and the resulting yellow suspension stirred at r.t. for 2.5 h. The reaction mixture was poured into ice-water (500 mL), saturated by the addition of solid NaCl, and then extracted with Et_2O (4 × 125 mL). The combined Et_2O extracts were successively washed with H_2O (× 2), sat. $NaHCO_3$, H_2O, and brine (125 mL each). Removal of the solvent gave 3.67 g of a pale yellow oil, which upon flash chromatography over silica gel (100 g) (CH_2Cl_2), furnished pure **15** (2.69 g, 80%) as a colorless oil. Further elution gave 0.75 g (14%) of substrate **14**.

^{1}H NMR ($CDCl_3$): δ = 7.40–7.33 (m, 5H, BnH), 5.12 (s, 2H, OCH_2Ph), 2.83 (t, 2H, *J* = 7.0, NCH_2), 2.37 (t, 2H, *J* = 7.5, CH_2CO), 1.69–1.35 [m, 6H, $(CH_2)_3$], 1.16 (s, 9H, CMe_3).

^{13}C NMR ($CDCl_3$): δ = 173.80, 136.37, 128.78, 128.42, 76.75, 66.21, 52.89, 34.30, 27.07, 26.92, 26.86, 24.93.

ESI MS: *m/z* = 294 $(M + H)^+$.

Anal. Calcd for $C_{17}H_{27}NO_3$: C, 69.59; H, 9.28; N, 4.77. Found: C, 69.73; H, 9.38; N, 4.92.

6-(*N*-Acetyl-*N*-*tert*-butyloxy)aminohexanoic Acid Benzyl Ester (16)

To a solution of hydroxylamine derivative **15** (1.03 g, 3.50 mmol) and pyridine (0.85 mL, 10.5 mmol) in CH_2Cl_2 (20 mL), protected from moisture by a $CaSO_4$ guard tube, was added acetic anhydride (0.66 mL, 7.0 mmol) at ice-bath temperature and the resulting mixture was stirred at r.t. for 3 h. The reaction mixture was poured into 50 g of crushed ice and allowed to stand for 1 h. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined CH_2Cl_2 extracts were washed successively with 1 N HCl, H_2O, sat. $NaHCO_3$, H_2O, and brine (30 mL each). Solvent removal followed by flash chromatography over silica gel (CH_2Cl_2–EtOAc, 9:1) afforded **16** (1.15 g, 98%) as a colorless viscous oil.

^{1}H NMR ($CDCl_3$): δ = 7.37–7.29 (m, 5H, BnH), 5.10 (s, 2H, OCH_2Ph), 3.59 (br m, 2H, NCH_2), 2.35 (t, 2H, *J* = 7.4, CH_2CO), 2.10 (s, 3H, CH_3CO), 1.71–1.61 (m, 4H, $CH_2CH_2CH_2$), 1.33–1.22 [m, 11H, CMe_3 (singlet at 1.29) and $CH_2CH_2CH_2$].

^{13}C NMR ($CDCl_3$): δ = 175.68, 173.37, 136.10, 128.54, 128.17, 82.55, 66.08, 49.81, 34.15, 27.73, 26.33, 25.97, 24.63, 21.54.

ESI MS: *m/z* = 336 $(M + H)^+$, 353 $(M + NH_4)^+$, 358 $(M + Na)^+$.

Anal. Calcd for $C_{19}H_{29}NO_4$: C, 68.03; H, 8.71; N, 4.18. Found: C, 67.92; H, 8.79; N, 4.31.

6-(*N*-Acetyl-*N*-*tert*-butyloxy)aminohexanoic Acid (17)

A pale yellow solution of *N*-acetyl ester **16** (1.14 g, 3.40 mmol,) in EtOH–1 N NaOH (40 mL; 9:1) was stirred at r.t. for 3.5 h. The bulk of EtOH was removed, the residue was dissolved in H_2O (40 mL) and extracted with Et_2O (2 × 30 mL). The aqueous layer was cooled in an ice-bath and the solution made acidic (pH ~3.0–4.0) by the dropwise addition of 3 N HCl (1.5 mL) while stirring. The aqueous layer was saturated by the addition of solid NaCl (10 g) and extracted with Et_2O (4 × 30 mL). The solvent was removed and the residue dried under high vacuum to give **17** (0.83 g, quantitative) as a very pale yellow viscous material.

^{1}H NMR ($CDCl_3$): δ = 3.60 (br m, 2H, NCH_2), 2.34 (t, 2H, *J* = 7.6, CH_2CO), 2.12 (s, 3H, CH_3CO), 1.70–1.60 (m, 4H, $CH_2CH_2CH_2$), 1.37–1.21 [m, 11H, CMe_3 (singlet at 1.30) and $CH_2CH_2CH_2$].

^{13}C NMR ($CDCl_3$): δ = 178.62, 176.03, 82.85, 49.75, 33.95, 27.76, 26.26, 25.95, 24.42, 21.39.

ESI MS: *m/z* = 246 $(M + H)^+$, 263 $(M + NH_4)^+$, 268 $(M + Na)^+$.

Anal. Calcd for $C_{12}H_{23}NO_4$: C, 58.75; H, 9.45; N, 5.71. Found: C, 58.49; H, 9.46; N, 5.69.

7,14-Bis(*tert*-butyloxy)-8,15-dioxo-7,14-diazahexadecanoic Acid Benzyl Ester (18)

A solution of acid **17** (0.83 g, 3.40 mmol), hydroxylamine derivative **15** (1.0 g, 3.4 mmol), and HATU (1.29 g, 3.40 mmol) in DMF (6.8 mL) was cooled in an ice-bath and DIPEA (1.18 mL, 6.80 mmol) was added with stirring. The resulting yellow solution was protected from moisture by a $CaSO_4$ guard tube and stirred at r.t. for 2 h. Most of the DMF was removed and the residue was partitioned between EtOAc and H_2O (40 mL each). The layers were separated and the aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic extracts were successively washed with sat. $NaHCO_3$, H_2O, 0.5 M $KHSO_4$, H_2O, and brine (30 mL each). The solvent was removed, and the residue (2.71 g) was subjected to flash chromatography over silica gel (CH_2Cl_2-EtOAc, 4:1) to furnish **18** (1.66 g, 94%) as a colorless viscous residue.

^{1}H NMR ($CDCl_3$): δ = 7.38–7.31 (m, 5H, BnH), 5.11 (s, 2H, OCH_2Ph), 3.60 (br m, 4H, 2 × NCH_2), 2.40 (br m, 2H, CH_2CO), 2.35 (t, 2H, *J* = 7.4, CH_2CO), 2.11 (s, 3H, CH_3CO), 1.70–1.56 (m, 8H, 2 × C*H*$_2$C*H*$_2$C*H*$_2$), 1.32−1.19 [m, 22H, 2 × CMe_3 (singlets at 1.30 and 1.29) and 2 × $CH_2CH_2CH_2$].

ESI MS: *m/z* = 521 $(M + H)^+$, 538 $(M + NH_4)^+$, 543 $(M + Na)^+$.

7,14-Bis(*tert*-butyloxy)-8,15-dioxo-7,14-diazahexadecanoic Acid (19)

A pale yellow colored solution of dimeric ester **18** (1.64 g, 3.15 mmol,) in EtOH–1 N NaOH (35 mL; 9:1) was stirred at r.t. for 3 h. Analytical TLC (CH_2Cl_2–EtOAc, 3:1) revealed the presence of a substantial amount of starting material. At this stage, another 1.2 mL of 1 N NaOH was added and the reaction mixture was stirred overnight (approx. 12 h) at r.t. The usual work up (CH_2Cl_2 was used for final extraction) followed by drying under high vacuum supplied **19** (1.30 g, 96%) as a colorless residue.

^{1}H NMR ($CDCl_3$): δ = 3.60 (br m, 4H, 2 · NCH_2), 2.42 (br m, 2H, CH_2CO), 2.35 (t, 2H, *J* = 7.1, CH_2CO), 2.14 (s, 3H, CH_3CO), 1.72–1.50 (m, 8H, 2 × C*H*$_2$C*H*$_2$C*H*$_2$), 1.38–1.26 [m, 22H, 2 × CMe_3 (singlets at 1.31 and 1.30) and 2 × $CH_2CH_2CH_2$].

ESI MS: *m/z* = 431 $(M + H)^+$, 448 $(M + NH_4)^+$.

7,14,21-Tris(*tert*-butyloxy)-8,15,22-trioxo-7,14,21-triazatriicosanoic Acid Benzyl Ester (20)

The reaction of dimeric acid **19** (1.29 g, 3.00 mmol) with hydroxylamine derivative **15** (0.88 g, 3.00 mmol) in the presence of HATU (1.14 g, 3.00 mmol) and DIPEA (1.04 mL, 6.00 mmol) in DMF (6.0 mL) was carried out for 2 h as described in the preparation of **18**. After a similar work up, the crude product (2.64 g) was subjected to flash chromatography over silica gel (CH_2Cl_2–EtOAc, 7:3) to give **20** (2.05 g, 97%) as a colorless viscous residue.

^{1}H NMR ($CDCl_3$): δ = 7.37–7.32 (m, 5H, BnH), 5.11 (s, 2H, OCH_2Ph), 3.60 (br m, 6H, 3 × NCH_2), 2.40 (br m, 4H, 2 × CH_2CO), 2.35 (t, 2H, *J* = 7.4, CH_2CO), 2.12 (s, 3H, CH_3CO), 1.71–1.50 (m, 12H, 3 × C*H*$_2$C*H*$_2$C*H*$_2$), 1.35–1.27 [m, 33H, 3 × CMe_3 (singlets at δ 1.30 for 9H and 1.29 for 18H) and 3 × $CH_2CH_2CH_2$].

ESI MS: *m/z* = 706 $(M + H)^+$, 723 $(M + NH_4)^+$.

7,14,21-Tris(*tert*-butyloxy)-8,15,22-trioxo-7,14,21-triazatriicosanoic Acid (21)

Hydrolysis of trimeric ester **20** (2.04 g, 2.89 mmol) with EtOH–1 N NaOH (45 mL, 9:1) for 15 h as described above in the preparation of **19**, afforded **21** (1.75 g, 98%) as a colorless residue, after the usual work up followed by drying under high vacuum.

^{1}H NMR ($CDCl_3$): δ = 3.65 (br m, 6H, 3 × NCH_2), 2.42 (unresolved triplet, 4H, 2 × CH_2CO), 2.34 (t, 2H, *J* = 7.1, CH_2CO), 2.12 (s, 3H, CH_3CO), 1.67–1.50 (m, 12H, 3 × C*H*$_2$C*H*$_2$C*H*$_2$), 1.37–1.24 [m, 33 H, 3 × CMe_3 (singlets at 1.31, 1.30, and 1.29) and 3 × $CH_2CH_2CH_2$].

^{13}C NMR ($CDCl_3$): δ = 177.84 (br), 177.15, 175.80, 82.61, 82.49, 82.43, 49.84 (br), 33.82, 32.97, 32.91, 27.71, 27.66, 26.59, 26.20, 26.10, 25.94, 24.50, 24.40, 21.38.

ESI MS: *m/z* = 616 $(M + H)^+$, 633 $(M + NH_4)^+$.

Anal. Calcd for $C_{32}H_{61}N_3O_8$: C, 62.41; H, 9.98; N, 6.82. Found: C, 62.32; H, 10.12; N, 6.83.

7,14,21-Trihydroxy-8,15,22-trioxo-7,14,21-triazatriicosanoic Acid (22)

A pale brown solution of the trimeric acid **21** (1.02 g, 1.65 mmol) in TFA (from a freshly opened bottle, 33 mL) was stirred at r.t. for 10 h while protected from moisture by a $CaSO_4$ guard tube. Most of the TFA was removed under high vacuum; the pale brown viscous residue was dissolved in CH_3CN (20 mL), and evaporated to dryness. The crude product was further dried under high vacuum to give 0.79 g of pale brown solid. Preparative chromatography over C_{18} silica gel (Bakerbond, 40 μm) (H_2O–CH_3CN, 7:3), followed by recrystallization from H_2O–CH_3CN (7:3) afforded 0.559 g (76%) of the pure product **22** as a white powder (in fact, the product precipitated from the column fractions and was collected by filtration and dried under high vacuum).

Mp 111–112 °C.

^{1}H NMR (DMSO-d_6): δ = 11.96 (br s, 1H, D_2O exchangeable, COOH), 9.67 (s, 1H, D_2O exchangeable, OH), 9.56 (s, 2H, D_2O exchangeable, OH), 3.46 (t, 6H, *J* = 6.9, 3 × NCH_2), 2.32 (t, 4H, *J* = 7.1, 2 × CH_2CO), 2.19 (t, 2H, *J* = 7.1, CH_2CO), 1.97 (s, 3H, CH_3CO), 1.55–1.43 (m, 12H, 3 × C*H*$_2$C*H*$_2$C*H*$_2$), 1.28–1.18 (m, 6H, 3 × $CH_2CH_2CH_2$).

^{13}C NMR (DMSO-d_6): δ = 174.41, 172.54, 170.08, 47.03, 46.93, 46.84, 33.58, 31.62, 26.22, 26.05, 25.96, 25.67, 24.18, 23.95, 20.33.

ESI MS: *m/z* = 448 $(M + H)^+$, 470 $(M + Na)^+$, 486 $(M + K)^+$; 446 $(M - H)^-$, 560 $(M + TFA)^-$.

Anal. Calcd for $C_{20}H_{37}N_3O_8$: C, 53.68; H, 8.33; N, 9.39. Found: C, 53.68; H, 8.42; N, 9.40.

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(21) *N*-Bis(4-nitrophenylsulfonyl)-*O*-(phenylmethyl)-hydroxylamine [^{1}H NMR (DMSO-d_6): δ = 10.90 (s, 1 H), 8.47 (d, 2 H, *J* = 8.8 Hz), 8.15 (d, 2 H, *J* = 8.8 Hz), 7.45–7.30 (m, 5 H), 4.94 (s, 2 H)] from **6a** and *O*-(1,1-Dimethylethyl)-*N*-(4-nitrophenylsulfonyl)hydroxylamine [^{1}H NMR (DMSO-d_6): δ = 10.21 (s, 1H), 8.46 (d, 2H, *J* = 8.8 Hz), 8.09 (d, 2H, *J* = 8.8 Hz), 1.17 (s, 9H)] from **6c**.
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(26) Compound with trace impurities was found to decompose at r.t. over time. Once purified, it can be stored in the refrigerator indefinitely.
(27) It is critical that the first 125-mL portion not be shaken, but rather gently mixed and then separated. This will ensure the separation of layers even after shaking well during all subsequent extractions.