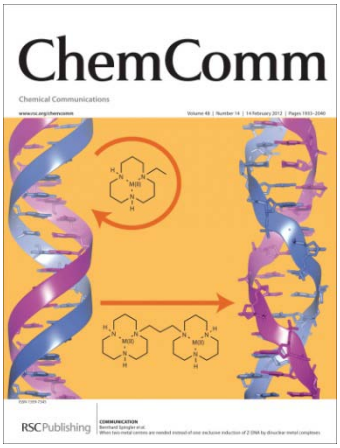
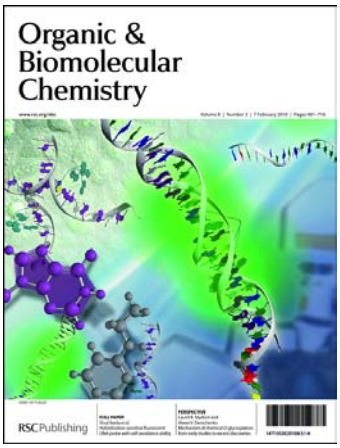




This article is part of the

Nucleic acids: new life, new materials

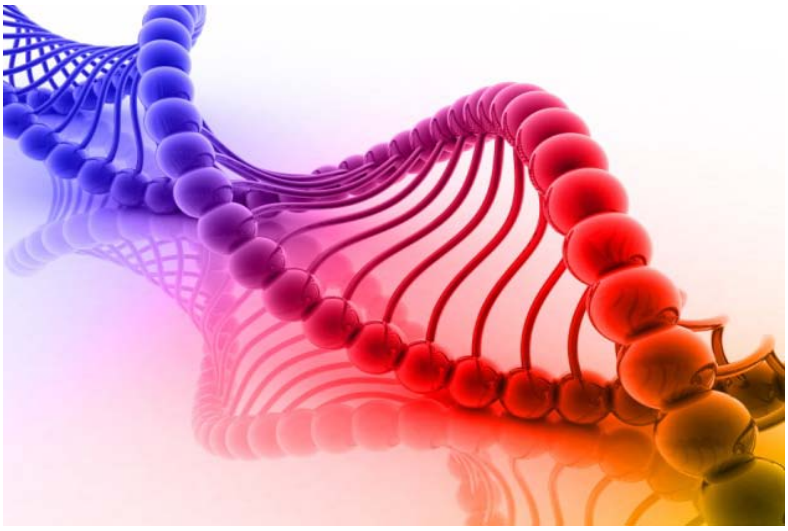
web-themed issue

Guest edited by:

Mike Gait Medical Research Council, Cambridge, UK	**Ned Seeman** New York University, USA	**David Liu** Harvard University, USA	**Oliver Seitz** Humboldt-Universität zu Berlin, Germany	**Makoto Komiyama** University of Tsukuba, Japan	**Jason Micklefield** University of Manchester, UK

All articles in this issue will be gathered online at

www.rsc.org/nucleic_acids



Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 8660

www.rsc.org/obc

PAPER

Concise and efficient syntheses of preQ$_1$ base, Q base, and (*ent*)-Q base†

Hans-Dieter Gerber and Gerhard Klebe*

Received 17th July 2012, Accepted 14th September 2012
DOI: 10.1039/c2ob26387d

To thoroughly study the functional role of prokaryotic t-RNA-guanine-transglycosylases which are essential in the pathogenesis of shigellosis, novel efficient, high-yielding synthetic approaches for preQ$_1$ base, Q base, as well as for (*ent*)-Q base mainly employing cheap and readily available starting materials have been developed. Q base as well as (*ent*)-Q base are accessible starting from preQ$_1$ base *via* nucleophilic substitution reactions with appropriately decorated halocyclopentenyl synthons, prior to that prepared from naturally occurring carbohydrates.

Introduction

t-RNA-guanine-transglycosylases (TGTs) are ubiquitously found in all three kingdoms of life. They are involved in the modification of tRNAs of numerous organisms including humans. In eukaryotic TGTs, the nucleobase queuine, also referred to as Q base (Fig. 1), is incorporated into cognate t-RNAs in the anticodon position 34 *via* a transglycosylation step.[1,2] In contrast, prokaryotic TGT, present in bacteria, is engaged in the hypermodification of t-RNAs that catalyze the base exchange reaction of guanine34 by the base preQ$_1$ (**1**) (Fig. 1), a precursor of queuine (**2**), which represents the 7-deaza-7-aminomethyl derivative of guanine.[3,4] Subsequently, in a biochemical pathway involving further enzymes, preQ$_1$ is chemically modified to reveal queuine.[2,5–15] This important difference in substrate specificity makes bacterial TGT an interesting drug target for the structure-based drug design of *e.g.* novel antibiotics. The respective shigella TGT enzyme was proven to play a crucial role in the regulation of the bacterial virulence of shigellosis, the causative agent of dysentery.[16,17] This bacterial infection is still responsible for more than one million deaths per year, especially affecting children in the developing countries.[18] Due to its essential role in the pathogenesis of dysentery, we have embarked on thorough investigations of this enzyme as a putative drug target.

To enable mutational studies of TGTs, in-depth enzyme-kinetic investigations, crystallization experiments with different enzyme mutants as well as analysis of the distinct substrate specificity of eukaryotic and prokaryotic TGTs, fairly large quantities of the above-described, natively occurring nucleobases are required.

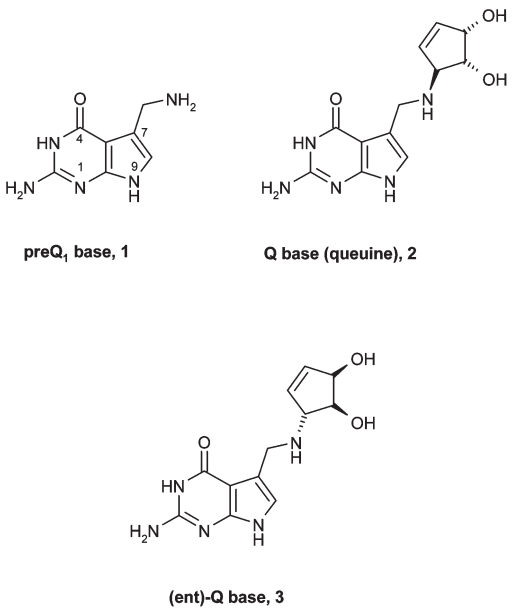


Fig. 1 Structures of preQ$_1$ base (numbering according to the nucleoside nomenclature), Q base, and (*ent*)-Q base.

In the literature a few synthetic routes for preQ$_1$ base (**1**) as well as for Q base (**2**) have been described as yet, including one for (*ent*)-Q base (**3**), which follows the corresponding Q base procedure described in the same paper.[19]

For preQ$_1$ base (**1**) as well as for Q base (**2**), four synthetic pathways have been reported so far, differing in length and synthetic effort from each other.[19–25]

A concise schematic overview (Schemes S1 and S2†) and discussion of the previously reported procedures for the synthesis of preQ$_1$ and Q bases can be found in the ESI.†

As all hitherto published syntheses for preQ$_1$ base (**1**) as well as Q base (**2**) are quite lengthy, low-yielding, or partially utilize

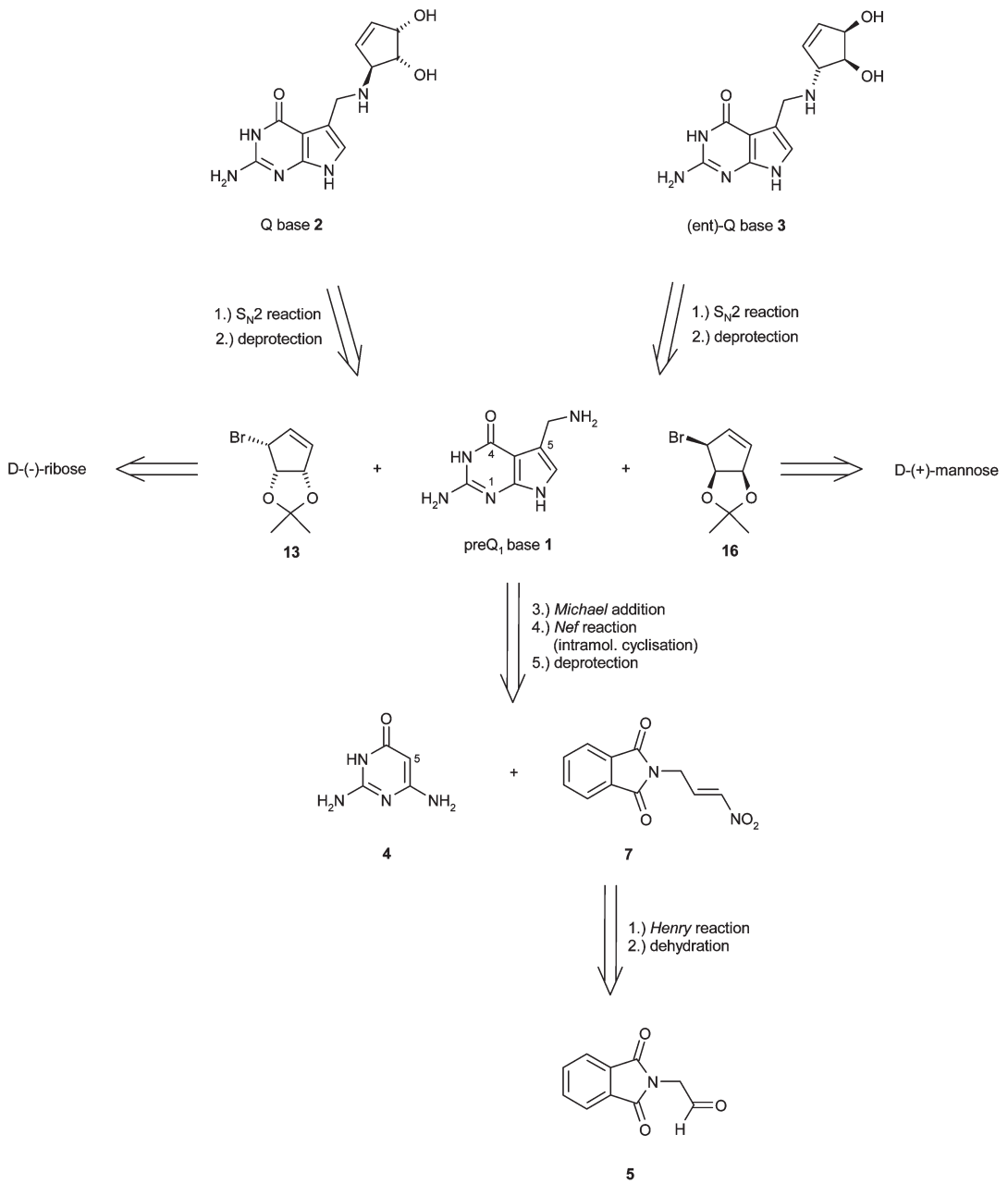
Institut für Pharmazeutische Chemie der Philipps-Universität Marburg, Marbacher Weg 6, 35032 Marburg, Germany.
E-mail: klebe@mailer.uni-marburg.de; Fax: (+)49-06421-2828994; Tel: (+)49-06421-2821313

†Electronic supplementary information (ESI) available: ^{1}H- and ^{13}C-NMR spectra of all compounds. See DOI: 10.1039/c2ob26387d

rather expensive chemicals, we sought for a more cost-saving, convenient approach aimed to mainly employ cheap and readily available starting materials, avoid laborious work-up protocols, and render the desired nucleobases in such amounts that would allow easier handling of successive synthetic operations thus offering access to other non-naturally occurring nucleobases such as (*ent*)-Q base (**3**) or related structures from a common intermediate.

As outlined in Scheme 1, for the synthesis of preQ$_1$ base we envisaged a *Michael addition* of the commercially available building block **4** to nitroolefin **7**, the latter designed as a functionalized, protected synthon for the pyrrolo[2,3*d*]pyrimidine-4-one formation as well as preQ$_1$ base side chain implementation. Synthon **7** was supposed to be conveniently accessible *via* a *Henry reaction* from the likewise commercially available phthalimidoacetaldehyde **5**. Subsequent *Nef reaction* of the heterocyclic *Michael adduct*, followed by intramolecular cyclization should furnish, after final phthalimide deprotection, the desired preQ$_1$ base (**1**) in large amounts merely utilizing readily available and cheap material.

Taking the expected convenient availability of considerable preQ$_1$ base quantities into account, Q base as well as (*ent*)-Q base were thought to be easily accessible from preQ$_1$ base as starting compound by applying just two additional steps involving an S_N2 reaction with the stereochemically appropriately decorated bromocyclopentene derivatives **13** and **16**, respectively



Scheme 1 Retrosynthetic analysis of preQ$_1$ base (systematically numbered), Q base, and (*ent*)-Q base.

(Scheme 1). Both synthons were supposed to be accessible *via* straightforward protocols starting from naturally occurring D-(−)-ribose and D-(+)-mannose, respectively.

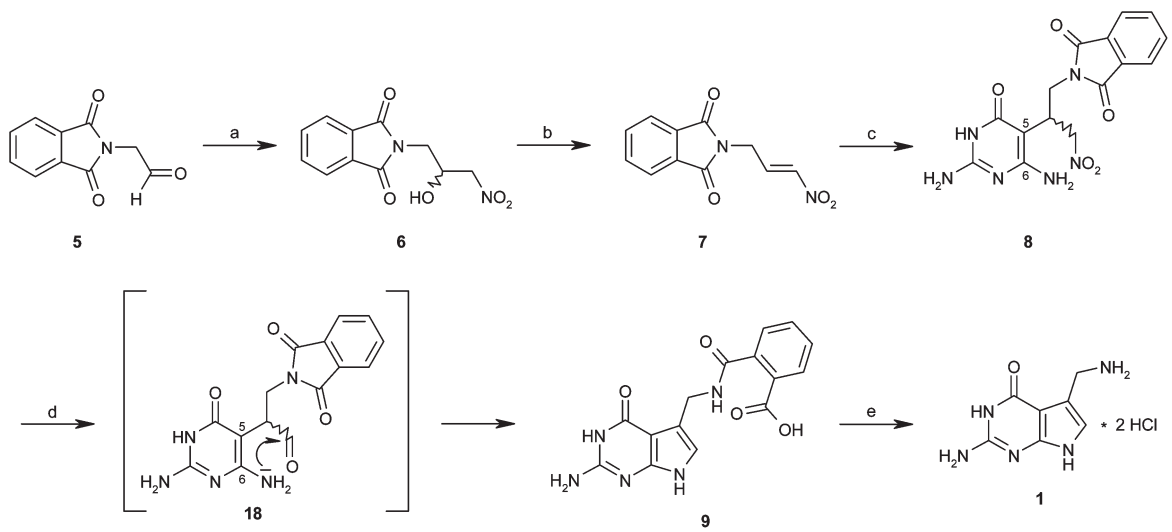
Results and discussion

Synthesis of preQ$_1$ base

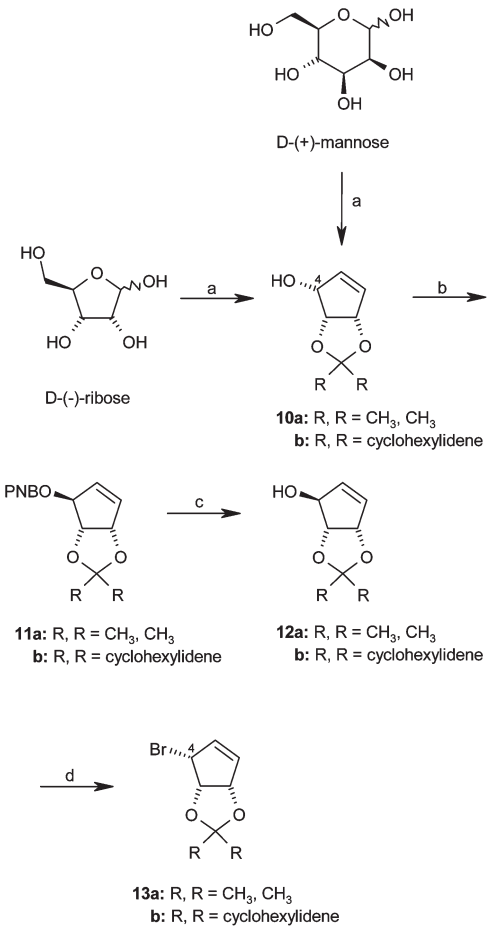
Our synthesis of preQ$_1$ base, outlined in Scheme 2, commenced with commercially available phthalimidoacetaldehyde (**5**), which was readily converted into racemic β-nitroalcohol **6** *via* a nitroaldol reaction (*Henry reaction*) in excellent yield. **6** was subsequently dehydrated under mild conditions *via* an intermediate hydroxyl group activation with trifluoroacetic anhydride, followed by trifluoroacetate elimination utilizing *N*,*N*-diisopropylethylamine[26] to yield nitroolefin **7** in good yield. The ^{1}H NMR spectrum of the raw product material revealed exclusive formation of one stereoisomer. The observed coupling constants (J = 13.5 Hz) indicated the presumed *trans* configuration of the corresponding olefinic protons thus confirming the yield of the *E*-isomer of **7**. Analogous to a procedure developed by Taylor *et al.*,[27] in the following step a *Michael addition* of pyrimidinone **4** to nitroolefin **7** involving the unsubstituted, quite nucleophilic position 5 of heterocycle **4** (Scheme 1) was easily performed by just stirring the starting compounds at 60 °C in a solvent mixture of THF–EtOAc–H_2O to readily afford racemic nitroalkyl derivative **8** in high yield and analytical purity after separation of the precipitated reaction product by simple filtration. The subsequently applied *Nef reaction* with **8** led to intermediate formation of aldehyde **18**, which directly underwent intramolecular cyclization thus forming the desired pyrrolo[2,3*d*]pyrimidine-4-one scaffold. However, this step happened to be the only one delivering a moderate product yield along the whole synthesis sequence. Interestingly, even at the rather low reaction temperatures (−5 °C to rt), semilateral hydrolysis of the phthalimido moiety was observed, obviously due to the particular reaction conditions present during the *Nef reaction*. Hence, this ring cleavage rendered the corresponding phthalamic acid derivative **9**, as indicated by ^{1}H NMR analysis of the isolated product. This finding prompted us to carry out removal of this altered *N*-protecting group under acidic reaction conditions utilizing aqueous hydrochloric acid in the final step. As a consequence, deprotection according to the *Ing-Manske* procedure,[28] which is usually employed for cleavage of the phthalimido protecting group, could be circumvented. The latter approach is frequently accompanied by major difficulties in product isolation resulting in tedious work-up procedures and quite often poor yields. Instead, in our case the acidic cleavage with hydrochloric acid performed excellently without any detectable side reaction product. Refluxing a suspension of pyrrolo[2,3-*d*]pyrimidin-4-one **9** in aqueous 6 M HCl led to neat truncation of the *N*-phthalimido protecting group, completeness of which was just indicated by a clear reaction solution usually obtained after 5–6 h of reflux. The ease of product isolation turned out to be a further important advantage gained by this approach. Separation of the resulting phthalic acid from the desired reaction product was simply achieved by complete removal of the volatile hydrochloric acid *in vacuo* and subsequent trituration of the remaining residue with diethyl ether which only dissolved the formed phthalic acid whereas the desired deprotected, doubly protonated product remained undissolved. Successive filtration, washing, and thorough drying of the obtained microcrystalline solid gave analytically pure preQ$_1$ base (**1**) as its dihydrochloride salt in almost quantitative yield.

Synthesis of Q base

As mentioned before, for the synthesis of the nucleobase queuine (**2**), preQ$_1$ base served as a starting point. In order to attach the required cyclopentenyl moiety to the aminomethyl side chain of preQ$_1$ base, an S_N2 reaction with the stereochemically appropriately decorated halocyclopentenyl precursor **13** as



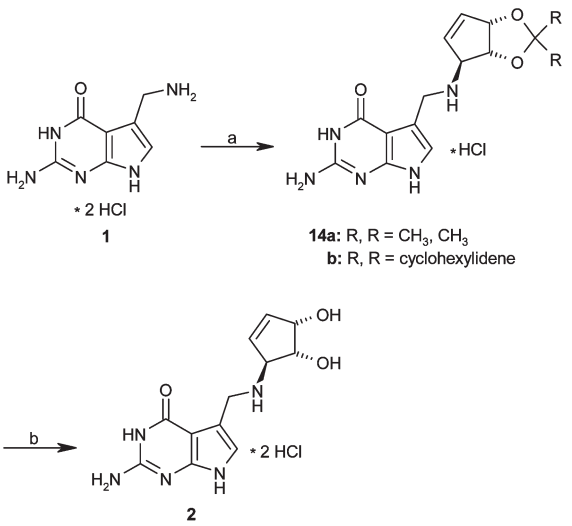
Scheme 2 Synthesis of preQ$_1$ base-dihydrochloride (**1**) reagents and conditions: (a) CH_3NO_2, cat. KO*t*Bu, THF/CH_3OH, 0–20 °C, 18 h, 93%; (b) TFAA, THF, −5 °C, 15 min, then TEA, THF, −10 °C, 5 min, 86%; (c) **4**, THF/EtOAc/H_2O, 60 °C, 4 h, 91%; (d) NaOH, H_2O, 20 °C, 5 min, then H_3O^+, H_2O, −5 °C to rt, 18 h, 47%; (e) 6 M HCl, reflux, 6 h, 98%.



Scheme 3 Synthesis of bromocyclopentene synthon **13** reagents and conditions: (a) for **10a**: 4 steps from D-(−) ribose, 23%,[29] for **10b**: 7 steps from D-(+)-mannose, 37%;[32] (b) PNBA, TPP, DEAD, THF, 55 °C, 48 h, for **11a**: 90%, for **11b**: 79%; (c) NaOH, MeOH–H_2O 5 : 1, 20 °C, 18 h, for **12a**: 93%, for **12b**: 96%; (d) Br_2, TPP, imidazole, CH_2Cl_2, −20 °C to rt, 1 h, for **13a**: 84%, for **13b**: 96%.

a substrate was envisioned, the synthesis of which is outlined in Scheme 3. Cyclopentenol **10a** was obtained in four steps with good overall yield applying a known procedure starting from naturally occurring D-(−)-ribose.[29]

According to a comparable literature protocol,[30] subsequent stereo inversion of the hydroxyl functionality of **10a** was easily accomplished *via* its conversion into 4-nitrophenylester **11a** under *Mitsunobu* conditions, saponification of which yielded the inverted alcohol **12a** in 84% over two steps. The following bromination under mild reaction conditions following a procedure of *Diederich et al.*[31] rendered bromocyclopentene **13a** in 84% yield. The resulting reinversion of the stereo centre at C-4 now provided the required configuration for the concluding S_N2 *reaction*. Alternatively, synthesis of cyclohexylidene-protected alcohol **10b** from D-(+)-mannose gave for almost all of the following reaction steps increased yields of the respective intermediates towards the synthesis of bromocyclopentene **13b** when compared to the corresponding acetonide-protected derivative **13a** thus leading to an improved overall yield (27% *versus* 16%) of this synthon.



Scheme 4 Synthesis of Q base-dihydrochloride **2** reagents and conditions: (a) **13a**, DBU, DMF, 65 °C, 5 h, 74%; **13b**, DBU, DMF, 65 °C, 5 h, 69%; (b) employing **14a**: 1.25 M HCl/MeOH, reflux, 5 h, 95%; employing **14b**: 1.25 M HCl/MeOH, reflux, 5 h, 91%.

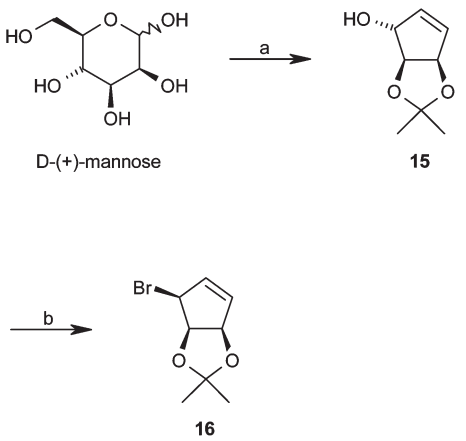
Q base side chain attachment

As depicted in Scheme 4, the final substitution reaction of bromocyclopentene **13a** or **b** with $preQ_1$ base-dihydrochloride was successfully carried out in DMF at 65 °C in the presence of DBU as a non-nucleophilic base. The crude product was subjected to column chromatography delivering a mixture of protected Q base as well as the resulting DBU-hydrohalides. Through trituration with chloroform and subsequent filtration of the resulting suspension, the latter side products could be removed rendering the desired protected Q base as monohydrochloride (**14a** or **b**). Acidic deprotection with 1.25 M HCl/MeOH finally rendered Q base (**2**) as dihydrochloride in 70% yield over two steps from $preQ_1$ base-dihydrochloride. For the purpose of kinetic studies to be carried out with Q base, it was very important to obtain a $preQ_1$-free product. Therefore, in these cases a moderate excess of **13a or b** was applied in the substitution reaction to ensure complete $preQ_1$ base consumption. A doubly-alkylated product, however, could not be observed. In this respect it should also be noted that employment of the cyclohexylidene-protected substrate **13b** turned out to be superior compared to the acetonide derivative **13a** delivering a particularly pure product.

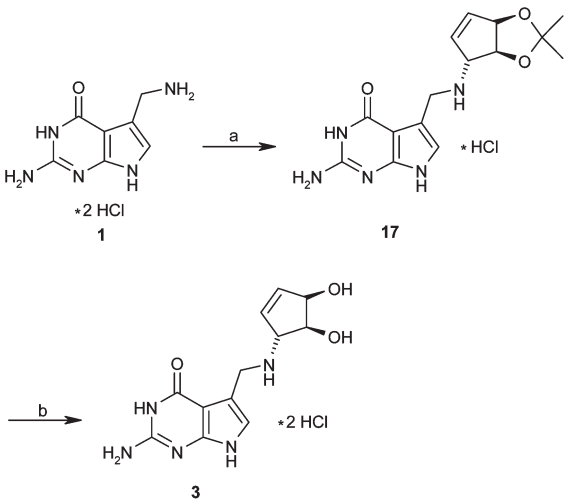
Synthesis of (*ent*)-Q base

In a similar manner, the Q base enantiomer (*ent*)-queuine (**3**) could also be synthesized (Scheme 6). The respective cyclopentenol **15** was accessible from D-(+)-mannose in 20.5% overall yield circumventing a *Mitsunobu* inversion at the hydroxyl function according to a high-yielding, straightforward literature protocol.[33] Subsequent bromination of **15** as described for alcohol **12** (Scheme 3) gave directly access to the desired enantiomeric bromocyclopentene **16** in 79% yield (Scheme 5).

Analogous to the synthesis of queuine, synthon **16** was reacted with $preQ_1$ base-dihydrochloride in the subsequent



Scheme 5 Synthesis of enantiomeric bromocyclopentene **16** reagents and conditions: (a) ref. 33; (b) Br_2, TPP, imidazole, −20 °C to rt, 1 h, 79%.



Scheme 6 Synthesis of (*ent*)-Q base-dihydrochloride **3** reagents and conditions: (a) **16**, DBU, DMF, 65 °C, 5 h, 67%; (b) 1.25 M HCl/MeOH, reflux, 5 h, 91%.

nucleophilic displacement rendering protected (*ent*)-Q base (**17**), which, after acidic deprotection, provided the stereochemically fully inverted isomer of Q base as dihydrochloride (**3**) in 61% yield over two steps (Scheme 6).

Conclusion

We have developed a short and straightforward, high-yielding synthetic pathway to $preQ_1$ base (**1**) which allows the convenient synthesis of this nucleobase on a gram-scale basis by a five-step approach (Scheme 2). In comparison to all hitherto published procedures, the overall yield (33.5% over five steps) could significantly be improved. Moreover, the sequence is based on inexpensive and readily available starting materials providing $preQ_1$ base in a highly favourable cost-benefit ratio.

In addition, we have worked out a strategy to readily synthesize significant amounts (>300 mg) of Q base (**2**) in just two additional steps from the starting compound $preQ_1$ base in 70% overall yield (Scheme 4). This approach circumvents a separate synthetic protocol for the heterocyclic core of Q base by applying an S_N2 reaction of $preQ_1$ base with the stereochemically appropriately decorated bromocyclopentene building block **13a**, the latter obtainable either starting from D-(−)-ribose or D-(+)-mannose (Scheme 3). Product yields for the building block **13** and consequently for Q base could further be improved when a cyclohexylidene protection (compounds **10b–13b**) instead of the commonly employed acetonide protection (compounds **10a–13a**) was applied.

In a similar fashion, the stereochemically fully inverted derivative of Q base, (*ent*)-Q base (**3**), was also synthesized (Scheme 6). The required corresponding enantiomeric bromocyclopentene **16** was prepared starting from D-(+)-mannose (Scheme 5).

In summary, the above reported strategies render the desired title compounds in high and overall improved yields by straightforward, convenient and low-cost approaches thus offering facile synthetic modifications at these nucleobases or other 7-deaza-7-aminomethylguanine-related derivatives.

Experimental section

General information

All proton and carbon NMR spectra were recorded on a JEOL ECA-500 MHz spectrometer (^{1}H NMR: 500.2 MHz, ^{13}C NMR: 125.8 MHz) or a JEOL ECX-400 MHz spectrometer (^{1}H NMR: 399.8 MHz, ^{13}C NMR: 100.5 MHz). The data were processed employing Delta NMR Processing and Control Software, version 4.3.6 or 5.0.0. Chemical shifts are stated in parts per million (ppm) and were referenced to TMS at 0.00 ppm for ^{1}H NMR spectra or to the solvent residual peak at 4.79 ppm for spectra in D_2O. For ^{13}C NMR spectra, references were adjusted to the following NMR solvent peaks: DMSO-d_6 at 39.5 ppm, CD_3OD at 49.0 ppm, $CDCl_3$ at 77.0 ppm. For D_2O spectra, CD_3OD was used as an internal reference and adjusted to 49.0 ppm. Abbreviations: bd = broad doublet, bs = broad singlet, d = doublet, dm = doublet of multiplets, dq = doublet of quartets, dt = doublet of triplets, m = multiplet, ps = pseudo, q = quartet, s = singlet, sm = symmetric multiplet, t = triplet. Mass spectra were obtained from a double-focussing sectorfield spectrometer type 7070 H (Vacuum Generators) or of type VG-Auto-Spec (Micromass). Combustion analyses were determined on a vario Micro cube CHNS analyzer (Elementar Analysensysteme GmbH) or a CHN autoanalyzer (Hewlett–Packard). Determination of chlorine was accomplished manually according to the Schöniger method. Optical rotations were obtained using a Jasco DIP-370 polarimeter at room temperature. Melting points were obtained by a melting point microscope HM-LUX (Leitz) and are uncorrected. Chromatography was performed using silica gel 60 (0.04–0.063 mm). TLC was carried out using 0.2 mm aluminium plates coated with silica gel 60 F_{254} and the products were visualized by UV detection or by utilization of phosphomolybdic acid ("blue stain"). Solvents and reagents that are commercially available were used in analytical quality without further purification unless otherwise noted. Tetrahydrofuran was dried by distillation from sodium/benzophenone. All moisture-sensitive reactions were carried out using oven-dried glassware under a positive stream of argon.

(*R*,*S*)-2-(2-Hydroxy-3-nitropropyl)-1*H*-isoindole-1,3(2*H*)-dione (6). To a stirred solution of phthalimidoacetaldehyde (**5**)[34] (18.92 g, 100.0 mmol) in THF (80 mL), nitromethane (9.12 g, 8.0 mL, 149.0 mmol, 1.5 equiv.) was added at ambient temperature. After cooling to 0–5 °C, a solution of KO*t*Bu (1.12 g, 10.0 mmol, 0.1 equiv.) in MeOH (30 mL) was added dropwise. The mixture was stirred for 60 min at 0–5 °C and then for 15 h at room temperature. After addition of 1 mL aq. HCl (32%), the mixture was evaporated *in vacuo* and the resulting residue thoroughly triturated with diethyl ether (250 mL). The precipitate was separated by filtration, washed with water, subsequently with diethyl ether, and dried *in vacuo* giving rise to analytically pure **6** (23.2 g, 93%) as almost a white solid. Mp: 185–186 °C (EtOAc–cyclohexane 2 : 1); ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ = 7.86 (sm, 4H), 5.77 (d, 1H, J = 5.3 Hz), 4.87 (psd, 1H, J = 10.1 Hz), 4.53–4.42 (m, 2H), 3.71 (dd, 1H, J = 14.0, 7.1 Hz), 3.60 (dd, 1H, J = 14.0, 5.3 Hz); ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ = 167.9, 134.3, 131.8, 123.0, 79.2, 65.7, 40.8; MS (ES+): m/z 273 (100, [M + Na]$^+$); HRMS (ES+) m/z calcd for $C_{11}H_{10}N_2O_5Na$: 273.0487, found: 273.0478; anal. calcd for $C_{11}H_{10}N_2O_5$ (250.21): C, 52.8; H, 4.0; N, 11.2; found: C, 53.0; H, 4.1; N, 11.4%.

2-[(2*E*)-3-Nitroprop-2-en-1-yl]-1*H*-isoindole-1,3(2*H*)-dione (7). To a stirred solution of **6** (7.50 g, 30.0 mmol) in dry THF (200 mL), TFAA (7.35 g, 4.9 mL, 35.0 mmol, 1.2 equiv.) in THF (5 mL) was carefully added at −5 °C under an argon atmosphere. After stirring for 15 min at this temperature, the mixture was cooled to −10 °C. *N*-Ethyl-*N*,*N*-diisopropylamine (12.0 mL, 70.0 mmol, 2.3 equiv.) was added at once, the resulting yellowish solution stirred for 5 min at −10 °C and then poured into MTBE (200 mL). The mixture was washed with a 10% NaH_2PO_4 solution (250 mL), and the aqueous layer was extracted with MTBE (2 × 50 mL). The combined organic layers were exhaustively washed with brine (3 × 100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was recrystallized (BuOAc–cyclohexane 7 : 3) yielding a first fraction of **7** (3.61 g, 52%) as pearly-coloured crystals. The mother liquid was evaporated *in vacuo*, dissolved in DMF (5 mL), and the solution was directly applied to a silica gel column. Chromatography with *iso*-hexane–EtOAc 3 : 2 (R_f = 0.65) gave rise to an additional fraction (2.37 g, 34%) of **7**. Mp: 131 °C (BuOAc–cyclohexane 7 : 3); ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ = 7.87 (sm, 4H), 7.61 (dt, 1H, J = 13.5, 1.8 Hz), 7.36 (dt, 1H, J = 13.5, 5.0 Hz), 4.48 (dd, 2H, J = 5.0, 1.8 Hz); ^{13}C NMR (DMSO-d$_6$, 125.8 MHz): δ = 167.3, 140.6, 137.9, 134.2, 131.9, 123.0, 35.3; MS (ES+) m/z 255 (39, [M + Na]$^+$); HRMS (ES+) m/z calcd for $C_{11}H_8N_2O_4Na$ 255.0381, found 255.0421; anal. calcd for $C_{11}H_8N_2O_4$ (232.20): C, 56.9; H, 3.5; N, 12.1; found: C, 56.8; H, 3.7; N, 12.1%.

(*R*,*S*)-2-[2-(2,4-Diamino-6-oxo-1,6-dihydropyrimidin-5-yl)-3-nitropropyl]-1*H*-isoindole-1,3(2*H*)-dione hydrate (8). Nitroolefin **7** (3.39 g, 14.6 mmol) and 2,6-diamino-3*H*-pyrimidin-4-one (**4**) (2.03 g, 16.1 mmol, 1.1 equiv.) were suspended in a solvent mixture of EtOAc (110 mL), THF (40 mL), and water (40 mL) and stirred at 60 °C. After 45 min, a beige-coloured precipitate started to form. The mixture was stirred for additional 3 h at 60 °C and then cooled to room temperature. The precipitate was separated by filtration, thoroughly washed with water, followed by EtOAc, and finally dried *in vacuo* to furnish analytically pure **8** (4.99 g, 91%) as monohydrate. Mp: 220–222 °C (EtOAc/THF/H$_2$O); ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ = 9.84 (bs, 1H), 7.83 (sm, 4H), 6.05 (bs, 2H), 5.79 (bs, 2H), 5.21 (dd, 1H, J = 12.6, 9.2 Hz), 4.91 (dd, 1H, J = 13.2, 5.7 Hz), 3.96 (dd, 1H, J = 13.8, 7.1 Hz), 3.75 (dd, 1H, J = 13.8, 5.0 Hz), 3.59 (bs, 1H); ^{13}C NMR (DMSO-d$_6$, 125.8 MHz): δ = 168.1, 162.6, 162.3, 153.7, 134.2, 131.7, 122.9, 83.2, 75.8, 38.1, 34.8; MS (ES+) m/z 359 (20, [M + H]$^+$); HRMS (ES+) m/z calcd for $C_{15}H_{15}N_6O_5$ 359.1104, found 359.1142; anal. calcd for $C_{15}H_{16}N_6O_6$ (376.33): C, 47.9; H, 4.3; N, 22.3; found: C, 48.0; H, 4.4; N, 22.7%.

2-({[(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)methyl]amino}carbonyl)benzoic acid dihydrate (9). Monohydrate **8** (3.58 g, 9.50 mmol) was dissolved in 2 M NaOH (30 mL, 60.0 mmol) and stirred for 5 min. The solution was filtered and the filtrate carefully added at −5 °C to a solution of 2.5 M H_2SO_4 (40 mL, 100.0 mmol). After stirring for 1 h at −5 °C, the reaction mixture was allowed to reach room temperature and stirred for additional 15 h. The resulting precipitate was dissolved at 0–5 °C by addition of 2.5 M NaOH (approx. 70 mL, pH 12), the solution filtered, and the filtrate carefully adjusted to pH 3 at 0–5 °C with 1 M HCl. The resulting precipitate was separated by filtration, the residue thoroughly washed with water, and dried *in vacuo* giving rise to dihydrate **9** (1.61 g, 47%) as a beige solid. Mp: > 240 °C (decomp.); ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ = 12.83 (bs, 1H), 10.84 (bs, 1H), 10.33 (bs, 1H), 8.81 (t, 1H, J = 5.5 Hz), 7.72 (dd, 1H, J = 7.6, 1.4 Hz), 7.58–7.44 (m, 3H), 7.50 (td, 1H, J = 7.6, 1.4 Hz), 7.50 (dd, 1H, J = 7.3, 1.4 Hz), 6.62 (m, 1H), 6.07 (bs, 2H), 4.48 (d, 2H, J = 5.3 Hz); ^{13}C NMR (DMSO-d$_6$, 125.8 MHz): δ = 168.3, 167.8, 159.8, 152.3, 151.6, 138.2, 131.4, 131.0, 129.3, 129.1, 127.5, 115.3, 113.9, 98.5, 35.9; MS (ES+) m/z 350 (100, [M + Na]$^+$); HRMS (ES+) m/z calcd for $C_{15}H_{14}N_5O_4$ 328.1046, found 328.1071; anal. calcd for $C_{15}H_{17}N_5O_6$ (363.33): C, 49.6; H, 4.7; N, 19.3; found: C, 49.8; H, 4.5; N, 19.5%.

2-Amino-5-(aminomethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one dihydrochloride hydrate (1). A suspension of dihydrate **9** (1.64 g, 4.5 mmol) in 6 M HCl (120 mL, 720 mmol) was refluxed until a clear solution was obtained (~6 h). The hot solution was stirred with charcoal, filtered, and the filtrate evaporated under reduced pressure to complete dryness. To the remaining residue diethyl ether (500 mL) was added and the resulting suspension triturated *via* sonication for 1 h, filtered, the residue thoroughly washed with diethyl ether, and dried *in vacuo*. The obtained microcrystalline product was finally dried at 80 °C/0.01 mbar to constant mass (~6 h) yielding light-brownish **1** (1.19 g, 98%). Mp: >250 °C (decomp.); ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ = 11.58 (bs, 2H), 8.36 (bs, 3H), 6.86 (s, 1H), 5.44 (bs, 3H), 4.03 (d, 2H, J = 5.5 Hz); ^{1}H NMR (CD$_3$OD, 500 MHz): δ = 6.97 (s, 1H), 4.21 (s, 2H); ^{1}H NMR (D$_2$O, 400 MHz): δ = 6.92 (s, 1H), 4.21 (s, 1H); ^{13}C NMR (DMSO-d$_6$, 125.8 MHz): δ = 158.6, 152.0, 145.0, 117.0, 111.2, 98.5, 34.7; ^{13}C NMR (D$_2$O, 125.8 MHz): δ = 160.6, 152.0, 142.8, 120.0, 111.7, 99.8, 36.0; MS (ES+) m/z 180 (90, [M + H]$^+$); HRMS (ES+) m/z calcd for $C_7H_{10}N_5O$ 180.0885, found

180.0867; anal. calcd for $C_7H_{13}Cl_2N_5O_2$ (270.12): C, 31.1; H, 4.85; N, 25.9; Cl, 26.25; found: C, 31.1; H, 4.6; N, 26.2; Cl, 26.0%.

(3′a*R*,4′*R*,6′a*S*)-4′,6′a-Dihydro-3′a*H*-spiro[cyclohexane-1,2′-cyclopenta[*d*][1,3]dioxole]-4′-ol (10b). Cyclopentenol **10b** was prepared according to a literature procedure for the enantiomeric form of the title compound.[32] The following ^{1}H NMR data of the colourless oil obtained after the final step matched with those of the enantiomer described in the literature. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.88 (m, 2H), 5.02 (dm, 1H, J = 5.50 Hz), 4.74 (t, 1H, J = 5.50 Hz), 4.55 (m, 1H), 2.79 (d, 1H, J = 9.9 Hz), 1.70–1.51 (m, 8H), 1.44–1.33 (m, 2H).

(3a*S*,4*S*,6a*S*)-2,2-Dimethyl-2*H*,3a*H*,4*H*,6a*H*-cyclopenta[*d*][1,3]-dioxol-4-yl 4-nitrobenzoate (11a). 4-Nitrophenylester **11a** was prepared according to a slightly modified procedure of *Seley et al.*[30] as following: to a solution of 4-nitrobenzoic acid (1.00 g, 6.0 mmol, 2.0 equiv.) and triphenylphosphine (1.57 g, 6.0 mmol, 2.0 equiv.) in 20 mL THF, diethyl azodicarboxylate (1.05 g, 6.0 mmol, 2.0 equiv.) in 5 mL THF was added dropwise and stirred at 20 °C for 30 min. Subsequently, a solution of cyclopentenol **10a**[29] (0.47 g, 3.0 mmol) in 5 mL THF was added and the mixture stirred at 55 °C for 48 h. The solvent was removed under reduced pressure, and the oily residue was purified by flash column chromatography on silica gel using EtOAc–*iso*-hexane 1 : 10 (R_f = 0.16) yielding 4-nitrophenyl ester **11a** (827 mg, 90%) as a colourless solid. Mp: 112–114 °C (EtOAc–*iso*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.29 (dt, 2H, J = 8.9, 2.1 Hz), 8.20 (dt, 2H, J = 8.9, 2.1 Hz), 6.24 (bd, 1H, J = 5.7 Hz), 6.03 (dm, 1H, J = 5.9 Hz), 5.88 (m, 1H), 5.35 (dq, 1H, J = 5.7, 0.9 Hz), 4.76 (d, 1H, J = 5.7 Hz), 1.47 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 164.1, 150.6, 138.3, 135.1, 130.80, 130.75, 123.5, 112.5, 84.7, 83.9, 83.2, 27.2, 25.6; MS (ES+) m/z 328 (100, $[M + Na]^+$); anal. calcd for $C_{15}H_{15}NO_6$ (305.29): C, 59.0; H, 4.95; N, 4.6; found: C, 59.0; H, 5.2; N, 4.8%; $[\alpha]_D^{20}$ +234° (c 0.98, MeOH).

(3a′*S*,4′*S*,6a′*S*)-4′,6a′-Dihydro-3a′*H*-spiro[cyclohexane-1,2′-cyclopenta[*d*][1,3]dioxol]-4′-yl 4-nitrobenzoate (11b). To a solution of 4-nitrobenzoic acid (3.34 g, 20.0 mmol, 1.6 equiv) and triphenylphosphine (5.25 g, 20.0 mmol, 1.6 equiv) in 60 mL THF, diazodiethylcarboxylate (3.48 g, 20.0 mmol, 1.6 equiv) in 10 mL THF was added dropwise and stirred at 20 °C for 30 min. Subsequently, a solution of cyclopentenol **10b** (2.50 g, 12.7 mmol) in 10 mL THF was added and the mixture stirred at 55 °C for 48 h. The solvent was removed under reduced pressure, and the remaining oily residue was purified by flash column chromatography on silica gel using EtOAc–*iso*-hexane 1 : 15 yielding 4-nitrophenyl ester **11b** (3.45 g, 79%) as a colourless solid. R_f = 0.28 (EtOAc–cyclohexane 1 : 15); Mp: 66–71 °C (*iso*-hexane); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.29 (dt, 2H, J = 8.9, 2.1 Hz), 8.20 (dt, 2H, J = 8.9, 2.1 Hz), 6.24 (d, 1H, J = 5.7 Hz), 6.02 (dm, 1H, J = 5.9 Hz), 5.88 (m, 1H), 5.35 (dq, 1H, J = 5.7, 0.9 Hz), 4.77 (d, 1H, J = 5.7 Hz), 1.72–1.52 (m, 8H), 1.46–1.34 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 164.1, 150.6, 138.6, 135.2, 130.8, 123.5, 113.3, 84.9, 83.6, 82.7, 37.0, 35.2, 25.0, 23.9, 23.7; MS (ES+) m/z 363 (100, $[M + NH_4]^+$); HRMS (ES+) calcd for $C_{18}H_{19}NO_6Na$ 368.1110, found 368.1126; $[\alpha]_D^{20}$ +123° (c 0.48, $CHCl_3$).

(3a*R*,4*S*,6a*S*)-2,2-Dimethyl-2*H*,3a*H*,4*H*,6a*H*-cyclopenta[*d*][1,3]-dioxol-4-ol (12a). Cyclopentenol **12a** was prepared according to a slightly modified procedure of *Seley et al.*[30] as following: nitrophenylester **11a** (823 mg, 2.7 mmol) was added to a solution of NaOH (1.00 g, 25.0 mmol, 9.3 equiv.) in 10 mL H_2O and 50 mL MeOH and stirred for 18 h at 20 °C. The reaction mixture was concentrated *in vacuo* and brine (20 mL) was added subsequently. After extraction with ethyl ether (3 × 50 mL), the combined organic layers were dried over $MgSO_4$, filtered, and the solvent removed *in vacuo* to afford spectroscopically pure **12a** (390 mg, 93%) as a colourless oil, which was directly used in the following reaction. R_f = 0.24 (EtOAc–*iso*-hexane 1 : 2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.04 (dm, 1H, J = 5.7 Hz), 5.92 (dm, 1H, J = 5.7 Hz), 5.29 (dq, 1H, J = 5.7, 0.9 Hz), 4.80 (bd, 1H, J = 4.8 Hz), 4.53 (d, 1H, J = 5.5 Hz), 1.94 (d, 1H, J = 5.9 Hz), 1.40 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 135.1, 134.7, 111.6, 85.8, 84.2, 80.6, 27.1, 25.6; $[\alpha]_D^{20}$ +112° (c 0.92, $CHCl_3$). All other analytical data were in accordance with those given in the literature.[35]

(3′a*R*,4′*S*,6′a*S*)-4′,6′a-Dihydro-3′a*H*-spiro[cyclohexane-1,2′-cyclopenta[*d*][1,3]dioxole]-4′-ol (12b). 4-Nitrophenylester **11b** (3.21 g, 9.3 mmol) was stirred at 20 °C for 18 h in a solution of NaOH (2.00 g, 50.0 mmol, 5.4 equiv) in 20 mL H_2O and 160 mL MeOH. The reaction mixture was concentrated *in vacuo* and brine (30 mL) was added to the solution. After extraction with ethyl ether (3 × 50 mL), the combined organic layers were dried over $MgSO_4$ and the solvent removed *in vacuo* to afford spectroscopically pure **12b** (1.75 g, 96%) as a colourless oil, which was directly used in the following reaction. R_f = 0.29 (EtOAc–cyclohexane 1 : 2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.04 (bd, 1H, J = 5.7 Hz), 5.90 (ddt, 1H, J = 5.7, 2.3, 0.9 Hz), 5.29 (dm, 1H, J = 5.6 Hz), 4.81 (dm, 1H, J = 6.0 Hz), 4.51 (d, 1H, J = 5.3 Hz), 1.90 (d, 1H, J = 6.2 Hz), 1.69–1.50 (m, 8H), 1.46–1.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 135.6, 134.6, 112.4, 85.5, 83.8, 81.1, 37.0, 35.1, 25.0, 24.0, 23.7; MS (EI) m/z 196 (51 $[M]^+$); HRMS (EI) m/z calcd for $C_{11}H_{16}O_3$ 196.1099, found 196.1105; $[\alpha]_D^{20}$ +132° (c 2.5, CH_3OH).

(3a*S*,4*R*,6a*S*)-4-Bromo-2,2-dimethyl-2*H*,3a*H*,4*H*,6a*H*-cyclopenta-[*d*][1,3]dioxole (13a). Compound **13a** was prepared according to a slightly modified procedure of *Diederich et al.*[31] as following: to a stirred solution of triphenylphosphine (525 mg, 2.0 mmol, 1.1 equiv) in CH_2Cl_2 (20 mL), bromine (320 mg, 2.0 mmol, 1.1 equiv) in CH_2Cl_2 (2 mL) was added dropwise at −10 °C. The resulting colourless solution was slowly added to a mixture of cyclopentenol **12a** (281 mg, 1.8 mmol) and imidazole (136 mg, 2.0 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) at −10 °C, the solution stirred for 45 min at −10 °C, and then allowed to reach room temperature. The reaction mixture was poured into TBME (50 mL), washed with water (2 × 20 mL), subsequently with brine (20 mL), filtered, dried over $CaCl_2$, and concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2 (2 mL) and the solution subsequently added dropwise to *iso*-hexane (50 mL). The precipitated TPPO was separated by filtration, thoroughly washed with *iso*-hexane, and the filtrate was concentrated *in vacuo*. Subsequent flash column chromatography of the oily residue with *iso*-hexane–EtOAc 15 : 1 (R_f = 0.45) yielded **13a** (331 mg, 84%) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 500 MHz):

δ = 5.98 (sm, 2H), 5.33 (sm, 1H), 4.97 (sm, 1H), 4.85 (sm, 1H), 1.39 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ = 135.1, 134.2, 112.2, 86.0, 84.1, 54.5, 27.4, 26.3; MS (EI) *m*/*z* 205 (92, $\{M - CH_3\}^+[^{81}Br]$), 203 (100, $\{M - CH_3\}^+[^{79}Br]$); HRMS (EI) calcd for $C_7H_8{}^{79}BrO_2$ 202.9708, found 202.9701; $[\alpha]_D^{20}$ −346° (*c* 0.39, $CHCl_3$).

(3a*S*,4*R*,6a*S*)-4-Bromo-4,6a-dihydro-3a*H*-spiro[cyclohexane-1,2-cyclopenta[*d*][1,3]dioxole (13b). To a stirred solution of triphenylphosphine (1.44 g, 5.5 mmol, 1.1 equiv) in CH_2Cl_2 (50 mL) was added bromine (880 mg, 5.5 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) at −10 °C. A mixture of cyclopentenol **12b** (981 mg, 5.0 mmol) and imidazole (408 mg, 6.0 mmol) was then added at −10 °C and the solution stirred for 18 h at 20 °C. The reaction mixture was poured into TBME (100 mL), washed with water and subsequently with brine, dried over $CaCl_2$, and evaporated to dryness. The residue was redissolved in CH_2Cl_2 (20 mL), and the solution was added dropwise to *iso*-hexane (100 mL). The resulting precipitate was separated by filtration, thoroughly washed with *iso*-hexane, and the filtrate concentrated *in vacuo*. *Iso*-hexane (30 mL) was added again and the solution separated from residual traces of TPPO. After removal of the solvent under reduced pressure and subsequent chromatography on silica gel with EtOAc–cyclohexane (1 : 10), **13b** (1.25 g, 96%) was obtained as a yellowish oil. R_f = 0.5 (EtOAc–cyclohexane 1 : 10); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.01–5.95 (m, 2H), 5.33 (dm, 1H, *J* = 5.5 Hz), 4.96 (d, 1H, *J* = 5.5 Hz), 4.86 (t, 1H, *J* = 1.8 Hz), 1.62–1.52 (m, 8H), 1.43–1.33 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 135.3, 134.1, 112.9, 85.5, 83.7, 54.8, 37.2, 35.7, 24.9, 23.9, 23.7; MS (EI) *m*/*z* 260 (14 $\{M\}^+[^{81}Br]$), 258 (18, $\{M\}^+[^{79}Br]$); HRMS (EI) *m*/*z* calcd for $C_{11}H_{15}{}^{79}Br\,O_2$ 258.0256, found 258.0239; $[\alpha]_D^{20}$ −325° (*c* 1.5, $CHCl_3$).

2-Amino-5({[(3a′*R*,4′*S*,6a′*S*)-2,2-dimethyl-4,6a′-dihydro-3a′*H*-cyclopenta[*d*][1,3]dioxol-4-yl]amino}methyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one hydrochloride (14a). To a suspension of **1** (378 mg, 1.4 mmol) in DMF (5 mL), DBU (2 mL, 13.4 mmol, 9.6 equiv.) was added and the mixture stirred at room temperature for 10 min. The resulting dark, clear solution was heated to 50 °C, after which a solution of protected bromocyclopentene **13a** (438 mg, 2.0 mmol, 1.4 equiv.) in DMF (1 mL) was added dropwise under an argon atmosphere. After stirring for 5 h at 65 °C, the reaction mixture was concentrated under reduced pressure. The residual dark brown oil was re-dissolved in MeOH (3 mL) and subjected to a silica gel column. Less-polar impurities were eluted starting with EtOAc, followed by a solvent mixture of EtOAc–MeOH–TEA 10 : 1 : 0.1. The product-containing fractions, which also consisted of the resulting DBU-hydrohalide salts, were obtained eluting with EtOAc–MeOH–TEA 3 : 1 : 0.01. Subsequent removal of the solvent gave rise to 550 mg of a beige solid which was triturated with $CHCl_3$ (150 mL) through stirring for 10 min. The suspension was filtered, the residue thoroughly washed with $CHCl_3$, and finally dried *in vacuo* rendering **14a** (367 mg, 74%) as almost a white solid. R_f = 0.21 (EtOAc–MeOH–TEA 3 : 1 : 0.01); Mp: >250 °C (MeOH, decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ = 6.88 (s, 1H), 6.30 (dt, 1H, *J* = 5.7, 1.4 Hz), 6.00 (dm, 1H, *J* = 5.7 Hz), 5.34 (dm, 1H, *J* = 5.7 Hz), 4.91 (d, 1H, *J* = 5.7 Hz), 4.39 (d, 1H, *J* = 13.7 Hz), 4.33 (d, 1H, *J* = 13.1 Hz), 4.31 (m, 1H), 1.37 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (CD_3OD, 125.8 MHz): δ = 162.7, 154.5, 154.0, 141.0, 128.7, 119.4, 113.6, 109.6, 99.8, 85.6, 81.5, 69.2, 43.4, 27.4, 25.7; MS (ES+) *m*/*z* 318 (100, $[M + H]^+$); HRMS (ES+) *m*/*z* calcd for $C_{15}H_{20}N_5O_3$ 318.1566, found 318.1561; $[\alpha]_D^{20}$ +88° (*c* 0.40, MeOH).

A small sample of the monohydrochloride **14a** was stirred with Na_2CO_3 in MeOH for 30 min, the suspension subsequently filtered, and finally evaporated to yield the free base as a white solid. ^{1}H NMR (CD_3OD, 400 MHz): δ = 6.59 (s, 1H), 5.90 (dt, 1H, *J* = 5.7, 1.4 Hz), 5.85 (dm, 1H, *J* = 5.7 Hz), 5.25 (dm, 1H, *J* = 5.7 Hz), 4.58 (d, 2H, *J* = 5.7 Hz), 3.91 (d, 2H, *J* = 13.3 Hz), 3.76 (m, 1H), 3.75 (d, 2H, *J* = 13.3 Hz), 1.33 (s, 3H), 1.32 (s, 3H).[19,24]

5-({[(3′a*R*,4′*S*,6′a*S*)-4′,6′a-Dihydro-3′a*H*-spiro[cyclohexane-1,2′-cyclopenta[*d*][1,3]dioxole]-4′-yl]amino}methyl)-2-amino-3*H*,4*H*,7*H*-pyrrolo[2,3-*d*]pyrimidin-4-one hydrochloride (14b). Following the procedure for the corresponding acetonide **14a**, reaction of **1** (540 mg, 2.0 mmol) with bromocyclopentene **13b** (490 mg, 2.5 mmol, 1.3 equiv) yielded 491 mg (69%) of **14b** as a white solid, which was further purified through recrystallization from MeOH. R_f = 0.24 (EtOAc–MeOH–TEA 3 : 1 : 0.01); Mp: >250 °C (MeOH, decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ = 6.88 (s, 1H), 6.31 (dm, 1H, *J* = 5.7 Hz), 5.99 (dm, 1H, *J* = 4.8 Hz), 5.33 (dm, 1H, *J* = 5.0 Hz), 4.40 (d, 2H, *J* = 13.7 Hz), 4.33 (d, 2H, *J* = 13.5 Hz), 4.33 (m, 1H), 1.66–1.53 (m, 8H), 1.45–1.35 (m, 2H); ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.28 (bs, 1H), 10.79 (bs, 1H), 9.38 (bs, 1H), 6.84 (s, 1H), 6.35 (bs, 2H), 6.29 (d, 1H, *J* = 5.5 Hz), 5.98 (d, 1H, *J* = 4.8 Hz), 5.27 (d, 1H, *J* = 5.0 Hz), 4.84 (d, 1H, *J* = 5.7 Hz), 4.24 (m, 3H), 1.60–1.43 (m, 8H), 1.39–1.27 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz): δ = 162.5, 154.4, 153.8, 139.4, 130.6, 118.8, 113.8, 111.9, 99.9, 85.3, 82.0, 69.4, 43.7, 38.1, 36.0, 26.1, 25.0, 24.8; MS (ES+) *m*/*z* 358 (100, $[M + H]^+$); HRMS (ES+) *m*/*z* calcd for $C_{18}H_{24}N_5O_3$ 358.1879, found 358.1872; $[\alpha]_D^{20}$ +94° (*c* 0.4, MeOH).

The free base of **14b** was obtained as described for the corresponding acetonide derivative **14a**. ^{1}H NMR (CD_3OD, 400 MHz): δ = 6.65 (s, 1H), 5.92 (dt, 1H, *J* = 5.7, 1.6 Hz), 5.84 (dm, 1H, *J* = 5.7 Hz), 5.23 (dq, 1H, *J* = 5.7, 0.7 Hz), 4.55 (d, 1H, *J* = 5.7 Hz), 3.92 (dd, 1H, *J* = 13.3, 0.7 Hz), 3.78 (dd, 1H, *J* = 13.3, 0.7 Hz), 3.77 (m, 1H,), 1.62–1.50 (m, 8H), 1.44–1.32 (bs, 2H); $[\alpha]_D^{20}$ +105° (*c* 0.3, MeOH).

2-Amino-5({[(1*S*,4*S*,5*R*)-4,5-dihydroxycyclopent-2-en-1-yl]amino}-methyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one dihydrochloride hydrate (2). Acetonide **14a** (353 mg, 1.0 mmol) was refluxed in a methanolic solution of 1.25 M HCl (60 mL) for 5 h. The solution was evaporated to complete dryness yielding **2** (349 mg, 95%) as almost a white solid. The corresponding cyclohexylidene-protected derivative **14b** yielded 334 mg (91%) of **2** from a 1.0 mmol reaction. Mp: >250 °C (decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ = 6.97 (s, 1H), 6.25 (dt, 1H, *J* = 6.2, 2.1 Hz), 6.06 (dd, 1H, *J* = 6.2, 1.6 Hz), 4.60 (dt, 1H, *J* = 5.7, 1.6 Hz), 4.55 (d, 1H, *J* = 13.8 Hz), 4.43 (d, 1H, *J* = 13.8 Hz), 4.31 (t, 1H, *J* = 5.7 Hz), 4.22 (sm, 1H); ^{1}H NMR (D_2O, 400 MHz): δ = 6.98 (s, 1H), 6.24 (dt, 1H, *J* = 6.4, 2.1 Hz), 6.06 (dd, 1H, *J* = 6.3, 1.6 Hz), 4.65 (dm, 1H, *J* = 5.7 Hz), 4.47 (d, 1H, *J* = 13.7 Hz), 4.39 (d, 1H, *J* = 13.7 Hz), 4.36 (t, 1H, *J* = 5.7 Hz), 4.26 (sm, 1H); ^{13}C NMR (CD_3OD, 125.8 MHz): δ = 161.0, 153.4, 145.8, 139.7, 129.8, 120.7,

110.6, 100.3, 75.2, 74.4, 68.5, 43.0; MS (ES+) *m/z* 278 (100, $[M + H]^+$); HRMS (ES+) *m/z* calcd for $C_{12}H_{16}N_5O_3$ 278.1253, found 278.1254; anal. calcd for $C_{12}H_{17}Cl_2N_5O_3{*}H_2O$ (368.22): C, 39.1; H, 5.2; N, 19.0; found: C, 39.3; H, 4.8; N, 19.1%; $[\alpha]_D^{20}$ +117° (*c* 0.30, H_2O).[26]

(3a*S*,4*R*,6a*R*)-2,2-Dimethyl-2*H*,3a*H*,4*H*,6a*H*-cyclopenta[*d*][1,3]-dioxol-4-ol (15). Cyclopentenol **15** was prepared according to a literature procedure.[33] R_f = 0.24 (EtOAc–*iso*-hexane 1 : 2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.04 (bd, 1H, *J* = 5.7 Hz), 5.91 (dm, 1H, *J* = 5.7 Hz), 5.29 (d, 1H, *J* = 5.5 Hz), 4.80 (bs, 1H), 4.53 (d, 1H, *J* = 5.7 Hz), 1.93 (d, 1H, *J* = 5.5 Hz), 1.40 (s, 3H), 1.35 (s, 3H); $[\alpha]_D^{20}$ −117° (*c* 0.98, $CHCl_3$). All other analytical data were in accordance with those given in the literature.

(3a*R*,4*S*,6a*R*)-4-Bromo-2,2-dimethyl-2*H*,3a*H*,4*H*,6a*H*-cyclopenta-[*d*][1,3]dioxole (16). Compound **16** was prepared according to the previously described procedure for the enantiomeric bromocyclopentene **13a**. 312 mg (79%) of a colourless oil were obtained from a 1.8 mmol reaction. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.99 (sm, 2H), 5.33 (sm, 1H), 4.97 (sm, 1H), 4.85 (sm, 1H), 1.39 (s, 3H), 1.36 (s, 3H); MS (EI) *m/z* 205 (83, $\{M - CH_3\}^+[^{81}Br]$), 203 (89, $\{M - CH_3\}^+[^{79}Br]$); HRMS (EI) calcd for $C_7H_8{}^{79}BrO_2$ 202.9708, found 202.9708; $[\alpha]_D^{20}$ +349° (*c* 0.96, $CHCl_3$).

2-Amino-5({[(3a′*S*,4′*R*,6a′*R*)-2,2-dimethyl-4,6a′-dihydro-3a′*H*-cyclopenta[*d*][1,3]dioxol-4-yl]amino}methyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one hydrochloride (17). Acetonide **17** was prepared according to the procedure for **14a**. 237 mg (67%) were obtained from a 1.0 mmol reaction. Mp: >250 °C (decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ = 6.89 (s, 1H), 6.30 (dt, 1H, *J* = 5.7, 1.6 Hz), 6.00 (dm, 1H, *J* = 5.7 Hz), 5.35 (dm, 1H, *J* = 5.7 Hz), 4.92 (d, 1H, *J* = 5.7 Hz), 4.40 (d, 1H, *J* = 13.8 Hz), 4.33 (d, 1H, *J* = 13.5 Hz), 4.32 (m, 1H), 1.37 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (CD_3OD, 125.8 MHz): δ = 162.6, 154.5, 154.0, 141.0, 128.7, 119.4, 113.6, 109.6, 99.7, 85.6, 81.5, 69.1, 43.3, 27.4, 25.7; MS (ES+) *m/z* 318 (100, $[M + H]^+$); HRMS (ES+) *m/z* calcd for $C_{15}H_{20}N_5O_3$ 318.1566, found 318.1561; $[\alpha]_D^{20}$ −92° (*c* 0.40, MeOH).

2-Amino-5({[(1*R*,4*R*,5*S*)-4,5-dihydroxycyclopent-2-en-1-yl]-amino}methyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one dihydrochloride hydrate (3). Compound **3** was prepared according to the procedure for **2**. From a 0.5 mmol reaction, **3** (168 mg, 91%) was obtained as almost a white solid. Mp: >250 °C (decomp.); ^{1}H NMR (CD_3OD, 400 MHz): δ = 6.98 (s, 1H), 6.25 (dt, 1H, *J* = 6.2, 2.1 Hz), 6.05 (dd, 1H, *J* = 6.4, 1.6 Hz), 4.61 (dm, 1H, *J* = 6.2 Hz), 4.52 (d, 1H, *J* = 14.0 Hz), 4.40 (d, 1H, *J* = 13.7 Hz), 4.30 (t, 1H, *J* = 5.7 Hz), 4.21 (sm, 1H); ^{1}H NMR (D_2O, 400 MHz): δ = 6.98 (s, 1H), 6.24 (ddd, 1H, *J* = 6.3, 2.5, 2.1 Hz), 6.06 (dd, 1H, *J* = 6.2, 1.4 Hz), 4.65 (dm, 1H, *J* = 5.7 Hz), 4.47 (d, 1H, *J* = 14.0 Hz), 4.39 (d, 1H, *J* = 14.2 Hz), 4.36 (t, 1H, *J* = 5.5 Hz), 4.26 (dq, 1H, 5.5, 1.6 Hz); ^{13}C NMR (CD_3OD, 125.8 MHz): δ = 161.2, 153.6, 139.8, 144.5, 129.8, 120.3, 110.6, 100.3, 75.5, 74.4, 68.5, 43.2; ^{13}C NMR (D_2O, 125.8 MHz): δ = 161.2, 152.6, 145.5, 138.8, 129.8, 120.9, 109.6, 99.8, 74.5, 73.9, 67.6, 42.6; MS (ES+) *m/z* 278 (100, $[M + H]^+$); HRMS (ES+) *m/z* calcd for $C_{12}H_{16}N_5O_3$ 278.1253, found 278.1281; $[\alpha]_D^{20}$ −111° (*c* 0.30, H_2O).

Acknowledgements

We thank Prof. Dr W. E. Diederich, Marburg, for many helpful discussions supporting this work.

Notes and references

1 R. K. Slany and H. Kersten, *Biochimie*, 1994, **76**, 1178–1182.
2 D. Iwata-Reuyl, *Bioorg. Chem.*, 2003, **31**, 24–43.
3 J. D. Kittendorf, L. M. Barcomb, S. T. Nonekowski and G. A. Garcia, *Biochemistry*, 2001, **40**, 14123–14133.
4 C. Romier, K. Reuter, D. Suck and R. Ficner, *Biochemistry*, 1996, **35**, 15734–15739.
5 T. Ritschel, C. d. Atmanene, K. Reuter, A. Van Dorsselaer, S. Sanglier-Cianferani and G. Klebe, *J. Mol. Biol.*, 2009, **393**, 833–847.
6 S. G. Van Lanen, J. S. Reader, M. A. Swairjo, V. de Crécy-Lagard, B. Lee and D. Iwata-Reuyl, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 4264–4269.
7 Y. Kuchino, H. Kasai, K. Nihei and S. Nishimura, *Nucleic Acids Res.*, 1976, **3**, 393–398.
8 J. S. Reader, D. Metzgar, P. Schimmel and V. de Crécy-Lagard, *J. Biol. Chem.*, 2004, **279**, 6280–6285.
9 R. Gaur and U. Varshney, *J. Bacteriol.*, 2005, **187**, 6893–6901.
10 S. D. Kinzie, B. Thern and D. Iwata-Reuyl, *Org. Lett.*, 2000, **2**, 1307–1310.
11 C. Grimm, R. Ficner, T. Sgraja, P. Haebel, G. Klebe and K. Reuter, *Biochem. Biophys. Res. Commun.*, 2006, **351**, 695–701.
12 M. Blaise, H. D. Becker, G. Keith, C. Cambillau, J. Lapointe, R. Giegé and D. Kern, *Nucleic Acids Res.*, 2004, **32**, 2768–2775.
13 V. Campanacci, D. Y. Dubois, H. D. Becker, D. Kern, S. Spinelli, C. Valencia, F. Pagot, A. Salomoni, S. Grisel, R. Vincentelli, C. Bignon, J. Lapointe, R. Giegé and C. Cambillau, *J. Mol. Biol.*, 2004, **337**, 273–283.
14 D. Y. Dubois, M. Blaise, H. D. Becker, V. Campanacci, G. Keith, R. Giegé, C. Cambillau, J. Lapointe and D. Kern, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 7530–7535.
15 J. C. Salazar, A. Ambrogelly, P. F. Crain, J. A. McCloskey and D. Söll, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 7536–7541.
16 J. M. B. Durand, B. Dagberg, B. E. Uhlin and G. R. Björk, *Mol. Microbiol.*, 2000, **35**, 924–935.
17 N. Okada and S. Nishimura, *J. Biol. Chem.*, 1979, **254**, 3061–3066.
18 K. L. Kotloff, J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak and M. M. Levine, *Bull. World Health Organ.*, 1999, **77**, 651–666.
19 H. Akimoto, H. Nomura, M. Yoshida, N. Shindo-Okada, A. Hoshi and S. Nishimura, *J. Med. Chem.*, 1986, **29**, 1749–1753.
20 T. Ohgi, T. Kondo and T. Goto, *Chem. Lett.*, 1979, **8**, 1283–1286.
21 H. Akimoto, E. Imamiya, T. Hitaka, H. Nomura and S. Nishimura, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1637–1644.
22 F. Klepper, K. Polborn and T. Carell, *Helv. Chim. Acta*, 2005, **88**, 2610–2616.
23 T. Kondo, T. Ohgi and T. Goto, *Chem. Lett.*, 1983, **12**, 419–422.
24 C. J. Barnett and L. M. Grubb, *Tetrahedron*, 2000, **56**, 9221–9225.
25 A. F. Brooks, G. A. Garcia and H. D. H. Showalter, *Tetrahedron Lett.*, 2010, **51**, 4163–4165.
26 S. E. Denmark and L. R. Marcin, *J. Org. Chem.*, 1993, **58**, 3850–3856.
27 E. C. Taylor and B. Liu, *Tetrahedron Lett.*, 1999, **40**, 4023–4026.
28 H. R. Ing and R. H. F. Manske, *J. Chem. Soc. (Resumed)*, 1926, 2348–2351.
29 F. Klepper, E.-M. Jahn, V. Hickmann and T. Carell, *Angew. Chem., Int. Ed.*, 2007, **46**, 2325–2327.
30 K. L. Seley, S. L. Mosley and F. Zeng, *Org. Lett.*, 2003, **5**, 4401–4403.
31 S. Brass, H.-D. Gerber, S. Dörr and W. E. Diederich, *Tetrahedron*, 2006, **62**, 1777–1786.
32 D. R. Borcherding, S. A. Scholtz and R. T. Borchardt, *J. Org. Chem.*, 1987, **52**, 5457–5461.
33 D. Vonlanthen and C. J. Leumann, *Synthesis*, 2003, 1090.
34 Commercially available from Aaron Chemistry GmbH, Am Fischweiher 41–43, 82481 Mittenwald, Germany.
35 H. Ovaa, B. Lastdrager, J. D. C. Codee, G. A. van der Marel, H. S. Overkleeft and J. H. van Boom, *J. Chem. Soc., Perkin Trans. 1*, 2002, 2370–2377.

