SELECTIVE REDUCTION OF THE 7-OXO GROUP IN PYRIDO[2,3-d]PYRIMIDINE-4,7-DIONES: A NEW SYNTHETIC APPROACH TO 5,10-DIDEAZATETRAHYDROFOLIC ACID (DDATHF)

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Abstract—A selective reduction of the carbonyl group at C7 with borane in THF is accomplished for 2,4-diamino-7-oxo and 2-amino-4,7-dioxo substituted pyridopyrimidines (7a-c) and (13a-c) respectively, the ester group remaining unaltered in both cases. The synthesis of 21c allows a new synthetic approach to 5,10-dideazatetrahydrofolic acid (DDATHF, 1) because it is a common intermediate in two procedures that afford the aforementioned antifolate.

5,10-Dideazatetrahydrofolic acid (DDATHF, 1) is a member of the second generation of antifolate analogues that overcomes, entirely or partially, the drawbacks associated with the administration of Methotrexate (MTX, 2) in the therapy of cancer. MTX presents, among others, a limited antitumoral spectrum and acquisition of drug resistance through four main mechanisms: membrane impaired transport, impaired polyglutamation, dihydrofolate reductase (DHFR, EC 1.5.1.3) increase activity, and decrease in the enzyme affinity. In contrast, DDATHF has a different mode of action respect to MTX, which inhibits DHFR, acting on glycaminide ribonucleotide transformylase (GARTFase, EC 2.1.2.2), responsible for the transformation of glycaminide ribonucleotide (GAR) into α-N-formylglycinamid ribonucleotide (FGAR), therefore inhibiting the biosynthesis of purines. To this respect DDATHF was the first antifolate clinically investigated with a locus of action different of DHFR and thymidilate synthase (TS, EC 2.1.1.45). The 6R-diastereomer of DDATHF (Lometrexol, LY264618), topological analogue of natural 6S-H₄FA, has recently finished Phase II clinical trials. 

![Chemical structures](image-url)
The first synthesis of DDATHF was described by Taylor in 1985 within a program focused on the preparation of deaza analogues of MTX. The synthetic pathway comprised fourteen steps and 0.94% global yield, employing extreme experimental conditions and reaction times. Since then Taylor et al. and other groups have described some new synthetic routes including an asymmetric one. The most efficient is a four-step convergent process with a 48% overall yield.

In this context, our group has synthesized a new family of 2,4-diamino-7-oxo and 2-amino-4,7-dioxo substituted tetrahydrofolic acid analogues of general structures (10a-c) and (16a-c), employing a novel strategy that implies the use of acyclic precursors (12a-c) and (11a-c) in contrast to the conventional use of preformed cyclic intermediates such as 6a-c (Scheme 1).

As it can be seen, the major difference between compounds (16a-c) and DDATHF is the presence of a carbonyl group at C-7 position. If reduction of this group would be possible, a new synthetic approach to DDATHF will be achieved.

RESULTS AND DISCUSSION

1 M Borane in tetrahydrofuran (Fluka part no. 15594) was chosen in order to assay the reduction of the extra carbonyl group at C7 due to its great selectivity for lactam groups with respect to other functional groups. At first sight, reduction of the carbonyl group could be carried out on the final products (16) as well as at intermediates (15), but the amide carbonyl group present in the side chain could interfere in the reaction. Consequently, esters (13a-c) were selected as substrates to assay the reduction because carboxylic acid groups present in 14a-c are incompatible with this reagent. Preliminarily, we successfully tested the reduction with BH$_3$THF on the 2,4-diamino-7-oxo substituted esters (7a-c), at 50°C for 72 h, to afford 17a-c in 79-94% yield, leaving unaltered the ester group.

However, the reduction of 15a-c had an added problem, the lactam carbonyl group at C-4, which also could be reduced with BH$_3$THF. No examples were found in the literature that implied the reduction of a 2-amino-4,7-dioxopyrido[2,3-d]pyrimidine. Consequently, we chose 18 as a model compound to study the behavior of the two oxo groups during the reduction of the pyrido[2,3-d]pyrimidine skeleton. Reaction was carried out employing 1 M BH$_3$THF at reflux for 24 h, with a 1:4.66 molar ratio of borane, to afford compound (19) in 80% yield. The most important conclusion obtained was to confirm the selective reduction of the 7-oxo group, the 4-oxo group remaining unaltered. It has to be pointed out that the solubility of 19 in organic solvents was greatly improved in respect to 18, supporting the idea that the great insolubility of 2-amino-7-oxo substituted pyrido[2,3-d]pyrimidines is a consequence of strong intermolecular H-bridge interactions caused by the polar front present in them.
Scheme 1

Spectroscopic data were of major importance in the evaluation of the results obtained. NMR especially gave the most useful and interesting information. Thus, the $^1$H-NMR spectrum showed two signals at 3.5 and 3.0 ppm corresponding to H-7 protons. The assignment of H-5 and H-7 was carried out by means of a H-C correlation spectrum. The $^{13}$C-NMR and DEPT spectra of 19 showed the absence of the signal at 179.4 ppm corresponding to the 7-oxo group and the presence of a signal at 49.3 ppm due to the new methylene unit. It is interesting to note the minimum variation in the chemical shift of C-4 (from 162.9 in 18 to 161.7 ppm in 19) and the shielding of C-4a (from 94.3 to 87.7 ppm) due to the greater donor effect of N-8. It is also noteworthy the shift to higher-field of C-6 (from 36.4 to 26.6 ppm) due to the absence of the deshielding effect of the 7-oxo group.
Taking this result into account, reduction was carried out on compounds (13a-c) in similar reaction conditions to afford 2-amino-4-oxopyrido[2,3-d]pyrimidines (20a-c) in 52-79% yields. If the destruction of the complex formed by borane and the reduced product with 6M HCl is prolonged more than the required time, carboxylic acids (21a-c) are obtained instead of 20a-c. Table 1 shows the variation of the chemical shifts in the $^{13}$C-NMR spectra of 13a-c in respect to 20a-c. Once again, C-7 and C-4a are shifted to higher-field.

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</table>

Table 1

To summarize, selective reduction of the carbonyl group at C7 with borane in THF is accomplished for 2,4-diamino-7-oxo and 2-amino-4,7-dioxo substituted pyridopyrimidines (7a-c) and (13a-c) respectively, the ester group remaining unaltered in both cases.

Moreover, the synthesis of 21c allows a new synthetic approach to DDATHF because it is a common intermediate in two procedures that afford the aforementioned antifolate. The first one is an asymmetric
synthesis which needs 16 steps to reach (R)-21c in 3.3 % yield. The second one, described by Taylor in 1996, consists in 7 steps with 38 % global yield in 6R-DDATHF. In our case, DDATHF can be obtained in 8 steps as the diastereomeric mixture of 6R-DDATHF (Lometrexol) and 6S-DDATHF with a non-optimized yield of 18 %.

ACKNOWLEDGMENTS

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EXPERIMENTAL

Compounds (7a-c), (13a-c) and (18) were prepared according to reported procedures. UV spectra were recorded on a Perkin-Elmer 683 and a Nicolet Magna 560 FTIR spectrophotometers. UV spectra were registered on a Hewlett-Packard 8450 spectrophotometer. NMR spectra were recorded on a Varian Gemini 300 spectrometer (300 and 75.5 MHz for 1H and 13C, respectively) using TSPNa as internal standard. MS spectra were obtained on a Hewlett-Packard 5995 A spectrometer. Elemental analyses were obtained on a Carlo-Erba CHNS-O/EA 1108 analyzer. Anhydrous THF was obtained by distillation over LiAlH4 and kept over 4 Å molecular sieves.

General procedure for the reduction of 7-oxo group present in 2,4-diamino-7-oxo substituted pyridopyrimidines (7). Methyl 4-[(2,4-Diamino-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-ylmethyl)methylamino]benzoate (17a). A suspension of 0.90 g (2.5 mmol) of 7a in 30 mL of anhydrous THF was heated to 50°C in an inert atmosphere. Then, 11.6 mL (11.6 mmol) of BH3·THF (1 M) were added dropwise with a syringe. During addition, hydrogen was evolved and the solid dissolved. After 72 h solvent was removed in vacuo, 10 mL of 6 M HCl were added to the crude material and reflux was maintained for 30 min. After cooling, the mixture was basified with 6M NaOH until pH 7-8. The solid formed was filtered off, washed with THF and dried in vacuum over P2O5 to give 0.81 g (94 %) of 17a as a white solid, mp 240-243 °C (MeOH). IR ν: 3350-3190 (N-H), 1692 (C=O), 1655, 1607, 1561 and 1527 (N-H, C=C, C=N), 765 (C-H). 1H NMR (TFA-d) δ: 2.36-2.42 (1H, m, H-5'), 3.84-3.88 (1H, m, H-5'), 3.45 (3H, s, OMe), 3.32-3.50 (1H, m, H-7'), 3.54-3.70 (1H, m, H-7), 3.82-3.94 (2H, m, N-CH2), 4.07 (3H, s, OMe), 7.86 (2H, m, H-3) and 8.40 (2H, m, H-2). 13C NMR (TFA-d) δ: 170.0 (COOMe), 154.0 (C4), 152.8 (C2'), 151.3 (C8a'), 145.0 (C4), 134.9 (C2), 134.7 (C1), 123.2 (C3), 80.3 (C4a), 62.8 (N-CH2), 55.2 (OMe), 49.3 (N-Me), 44.8 (C7'), 28.7 (C6') and 23.7 (C5'). MS, m/z (%): 342 (26) [M]+, 311 (15), 283 (11), 178 (74), 164 (25). UV (MeCN): λmax (log ε): 314 (4.33). Anal. Calcd for C17H10N5O2: C, 59.63; H, 6.48; N, 24.54. Found: C, 59.42; H, 6.50; N, 24.43.

Methyl 4-[(2,4-Diamino-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-ylmethyl)amino]benzoate (17b). The same procedure as described above but using: 0.20 g (0.60 mmol) of 7b and 3.6 mL (3.6 mmol) of BH3·THF (1M) in 4 mL of THF to give 0.17 g (94%) of 17b as a white solid, mp 176-178 °C (MeOH). IR ν: 3350, 3200 (N-H), 1700 (C=O), 1660, 1610 and 1570 (N-H, C=C, C=N), 750 (C-H). 1H NMR (TFA-d) δ: 2.56 (1H, b. s., H-5'), 2.83 (1H, b. s., H-5'), 2.91 (1H, b. s., H-6'), 3.57-3.85 (4H, m, H-6 and H-7'), 4.06 (3H, s, OMe), 7.40 (2H, m, H-3) and 8.30 (2H, m, H-2). 13C NMR (TFA-d) δ: 170.3 (COOMe), 154.0
Methyl 4-[2-(4-Diamino-5,6,7,8-tetrahydropyrido[2,3-d]pyrimidin-6-yl)ethyl]benzoate (17c). The same procedure as described above but using: 1.0 g (2.9 mmol) of 7c and 13.5 mL (13.5 mmol) of BH$_3$·THF (1 M) in 30 mL of THF to give 0.76 g (79%) of 17c as a white solid, mp 252-254 °C (MeOH). IR ν: 3400-3150 (N–H), 1750 (C=O), 1650, 1605 and 1585 (N–H, C=C, C=N), 760 (C–H). $^1$H NMR (TFA-d) δ: 1.70-1.89 (2H, m, Ar-CH$_2$-CH$_2$), 1.90-2.10 (1H, m, H-6’), 2.16 (1H, dd, J=15 Hz and J=10 Hz, H-5’), 2.70 (1H, dd, J=15 Hz and J=5 Hz, H-5’), 2.75-2.91 (1H, m, Ar-CH$_2$), 3.19 (1H, dd, J=13 Hz and J=10 Hz, H-7’), 3.67 (1H, dd, J=13 Hz and J=3 Hz, H-7), 3.99 (3H, s, OMe), 7.29 (2H, m, H-3) and 7.97 (2H, m, H-2). $^{13}$C NMR (TFA-d) δ: 173.3 (COOme), 153.0 (C4’), 152.2 (C2’), 151.1 (C8’a), 149.9 (C4), 134.0 (C2), 130.2 (C3), 128.8 (C1), 82.3 (C4’a), 54.5 (OMe), 47.4 (C7’), 35.3 (Ar-CH$_2$-CH$_2$), 34.3 (Ar-CH$_2$), 31.1 (C6’) and 25.4 (C5’). MS, m/z (%): 327 (100) [M$^+$], 178 (13), 164 (33), 162 (23), 150 (50). UV (MeCN): λ$_{max}$ (log ε): 284 (4.07). Anal. Calcd for C$_{27}$H$_{25}$N$_5$O$_2$: C, 62.37; H, 6.47; N, 21.39. Found: C, 62.39; H, 6.45; N, 21.40.

2-Amino-6-methyl-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine (19). A suspension of 0.5 g (2.6 mmol) of 2-amino-6-methyl-4,7-dioxo-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidine (18) in 4 mL of anhydrous THF was heated at reflux in an inert atmosphere. Then, 1.2 mL (12.1 mmol) of 1 M BH$_3$·THF were added dropwise with a syringe. During addition hydrogen was evolved and the solid dissolved. After 24 h at reflux the mixture was allowed to cool, solvent was eliminated in vacuo and the solid residue was suspended in 5 mL of 6 M HCl and refluxed for 30 min. After cooling, the resulting mixture was basified at pH 7-8 with 6 M NaOH. The solid was collected, recrystallized from glacial acetic acid, filtered off, washed with water and dried over P$_2$O$_5$ to give 0.37 g (80% yield) of 19 as a white solid, mp 261-263 °C. IR ν: 3450-3000 (N–H), 1670 (C=O), 1635, 1600, 1570 and 1550 (N–H, C=C, C=N). $^1$H NMR (TFA-d) δ: 1.11 ppm (3H, d, J=6 Hz, Me), 1.99-2.10 (1H, m, H-6), 2.10 (1H, dd, J=11 Hz, J=11 Hz, H-5’), 2.77 (1H, dd, J=11 Hz, J=2 Hz, H-5) 3.08 (1H, dd, J=14 Hz, J=9 Hz, H-7) and 3.57 (1H, dd, J=14 Hz, J=4 Hz, H-7). $^{13}$C NMR (TFA-d) δ: 161.7 (C4), 154.4 (C2), 151.7 (C8’a), 87.7 (C4’a), 49.3 (C7’), 26.9 (C5), 26.6 (C6) and 18.0 (Me). MS, m/z (%): 180 (85) [M$^+$], 165 (100), 151 (15), 110 (21). UV (CH$_3$CN): λ$_{max}$(log ε): 283 (4.02), 224 (4.27). Anal. Calcd for C$_9$H$_{14}$N$_2$O·0.3H$_2$O: C, 51.77; H, 6.84; N, 30.18. Found: C, 52.18; H, 6.72; N, 29.85.

General procedure for the reduction of 7-oxo group in 2-amino-4,7-dioxo substituted pyrido[2,3-d]pyrimidines (13). Methyl 4-[[2-amino-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidin-6-ylmethyl]amino]benzoate (20a). A suspension of 0.5 g (1.4 mmol) of 13a in 10 mL of anhydrous THF was heated at 50°C in an inert atmosphere. Then, 6.5 mL (6.5 mmol) of 1 M BH$_3$·THF were added dropwise with a syringe and the heating was maintained for 72 h. Solvent was eliminated in vacuo and the residue was heated at reflux with 10 mL of 6 M HCl for 30 min. After cooling, the mixture was basified to pH 7-8 with 6 M NaOH. The resulting solid was filtered off, washed with THF and recrystallized from hexane/ethanol to give 0.25 g (52% yield) of 20a as a white solid, mp >250 °C. IR ν: 3400-3000 (N–H), 1680 (C=O), 1640, 1595 and 1510 (N–H, C=C, C=N), 750 (C–H). $^1$H NMR (TFA-d) δ: 2.10-2.41 (1H, m, H-5’), 2.40-2.60 (1H, m, H-6’), 2.60-2.86 (1H, m, H-5’), 3.48 (3H, s, N-Me), 3.30-3.85 (4H, m, H-7’ and N-CH$_2$), 4.07 (3H, s, OMe), 7.87 (2H, m, H-3) and 8.35 (2H, m, H-2). $^{13}$C NMR (TFA-d) δ: 170.2 (COOme), 163.2 (C4’), 152.8 (C2’), 152.1 (C8’a), 145.2 (C4), 135.0 (C2), 134.8 (C1), 123.4 (C3), 85.4 (C4’a), 63.4 (N-CH$_2$), 55.4 (OMe), 49.7 (N-Me), 45.1 (C7’), 28.9 (C6’) and 23.7 (C5’). MS, m/z
Methyl 4-[[2-amino-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidin-6-ylmethyl]amino]benzoate (20b). The procedure was the same as that stated above for 20a but using 1.0 g (2.9 mmol) of 13b in 30 ml of THF, 17.4 ml (17.4 mmol) of 1 M BH₃·THF and 20 ml of 6 M HCl to afford 0.71 g (74% yield) of 20b as a white solid, mp >250 °C (hexane/ethanol). IR ν: 3330-3000 (N-H), 1692 and 1663 (C=O), 1632, 1606 and 1528 (N-H, C=O, C=N), 770 (C-H). ¹H NMR (TFA-d) δ: 2.54 (1H, dd, J=16 Hz, J=7 Hz, H-5), 2.64-2.78 (1H, m, H-6'), 2.92 (1H, dd, J=16 Hz, J=4 Hz, H-5'), 3.46 (1H, dd, J=13 Hz, J=7 Hz, H-7'), 3.67-3.80 (3H, m, H-7' and N-CH₂), 4.06 (3H, s, OMe), 7.74 (2H, m, H-3) and 8.29 (2H, m, H-2). ¹³C NMR (TFA-d) δ: 170.3 (COOMe), 163.2 (C4'), 152.8 (C2'), 152.0 (C8a'), 140.0 (C4), 134.2 (C2), 133.9 (C1), 124.6 (C3), 84.9 (C4a'), 56.9 (N-CH₃), 55.0 (OMe), 44.8 (C7'), 29.2 (C6') and 23.3 (C5'). MS, m/z (%): 329 (24) [M⁺], 298 (10), 178 (59), 165 (100), 164 (37), 151 (21). Anal. Calcd for C₁₉H₁₅N₅O₅: C, 58.35; H, 5.81; N, 21.26. Found: C, 58.07; H, 5.72; N, 21.04.

Methyl 4-[[2-amino-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidin-6-yl]ethyl]benzoate (20c). The procedure was the same as that stated above for 20a but using 1.0 g (2.9 mmol) of 13c in 40 ml of THF, 13.5 ml (13.5 mmol) of 1 M BH₃·THF and 25 ml of 6 M HCl to afford 0.76 g (2.3 mmol, 79% yield) of 20c as a white solid, mp >250 °C (hexane/ethanol). IR ν: 3300-3000 (N-H), 1690 and 1660 (C=O), 1640, 1610 and 1520 (N-H, C=O, C=N), 745 (C-H). ¹H NMR (TFA-d) δ: 1.66-1.82 (2H, m, Ar-CH₂-CH₃), 1.80-2.00 (1H, m, H-6), 2.11-2.20 (1H, m, H-5'), 2.66-2.87 (3H, m, H-5' and Ar-CH₃), 3.10-3.20 (1H, m, H-7'), 3.63 (1H, d, J=14 Hz, H-7'), 3.99 (3H, s, OMe), 7.29 (2H, m, H-3) and 7.96 (2H, m, H-2). ¹³C NMR (TFA-d) δ: 173.2 (COOMe), 161.8 (C4'), 154.0 (C2'), 151.4 (C8a'), 150.0 (C4), 131.8 (C2), 130.1 (C3), 128.5 (C1), 87.1 (C4a'), 54.3 (OMe), 47.4 (C7'), 35.1 (Ar-CH₂-CH₃), 34.2 (Ar-CH₃), 31.0 (C6') and 25.0 (C5'). Anal. Calcd for C₁₉H₁₅N₅O₅: C, 62.18; H, 6.14; N, 17.06. Found: C, 62.16; H, 6.16; N, 17.05.

4-[[2-Amino-4-oxo-3,4,5,6,7,8-hexahydropyrido[2,3-d]pyrimidin-6-yl]ethyl]benzoic acid (21c). The same procedure as described above for 20c but using: 0.50 g (1.5 mmol) of 13c, 7.0 ml (7.0 mmol) of 1 M BH₃·THF in 20 ml of THF. Destruction of the complex was carried out with 10 ml of 6 M HCl during several hours to afford 0.31 g (65% of 21c, mp >200 °C ([R]-21c, lit. mp 312 °C). IR ν: 3300-2700 (N-H, O-H), 1680 (C=O), 1635, 1605 and 1515 (N-H, C=O, C=N), 730 (C-H). ¹H NMR (TFA-d) δ: 1.70-1.86 (2H, m, Ar-CH₂-CH₃), 1.86-2.02 (1H, m, H-6), 2.18 (1H, dd, J=16 Hz, J=10 Hz, H-10), 2.76-2.90 (3H, m, H-5' and Ar-CH₂), 3.15 (1H, dd, J=13 Hz, J=9 Hz, H-7'), 3.66 (1H, dd, J=13 Hz, J=2 Hz, H-7), 7.33 (2H, m, H-3) and 8.03 (2H, m, H-2). ¹³C NMR (TFA-d) δ: 175.6 (COOH), 161.7 (C4'), 154.2 (C2'), 151.5 (C8a'), 151.0 (C4), 132.6 (C2), 130.3 (C3), 127.6 (C1), 87.3 (C4a'), 47.7 (C7'), 35.2 (Ar-CH₂-CH₃), 34.4 (Ar-CH₂), 31.1 (C6') and 25.1 (C5'). MS, m/z (%): 193 (2), 179 (3), 165 (3), 149 (4), 135 (4), 57 (29), 43 (52). UV (MeCN): λmax(log ε): 282 (4.00), 225 (4.40).

REFERENCES


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